EFFECT OF SORGHUM FLOUR COMPOSITION AND PARTICLE SIZE ON QUALITY OF GLUTEN-FREE BREAD

By

EMILY J. FREDERICK

B.S., KANSAS STATE UNIVERSITY, MANHATTAN, KANSAS, 2007

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science Program

College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2009

Approved by:_____
Co-Major Professor
Dr. Fadi Aramouni
Dept. of Animal Sciences & Industry

Approved by:_____
Co-Major Professor
Dr. Jeff Gwirtz
Dept. of Grain Science & Industry

ABSTRACT

In order to improve the quality of products available for consumers who require a gluten-free diet, this study examined the effects of sorghum flour composition and particle size on functionality in gluten-free batter bread. White, food-grade sorghum was milled to flour of varying extraction rates (60%, 80%, 100%), and was subsequently pin-milled at different speeds (no pin-milling, low- speed, and high-speed) to create flours of both variable composition and particle size. Two commercially-milled sorghum flour samples (AF and TV) were included in the study and subjected to the same pin-milling treatments. Characterization of each flour included measurements of flour composition, total starch content, particle size distribution, damaged starch, and water absorption. Bread characterization included measurement of specific volume, crumb properties, and crumb firmness through the use of digital imaging and texture profile analysis.

Significant differences were found ($p < 0.05$) in the composition of sorghum flours of varying extraction rate, most notably for fiber and total starch contents. Flour particle size and starch damage were significantly impacted by extraction rate and speed of pin milling ($p < 0.05$). With the exception of the commercial flour samples, water absorption increased significantly with increasing extraction rate and speed of pin-milling speed ($p < 0.05$).

Within all treatments, breads produced from 60% extraction flour had significantly higher specific volumes, better crumb properties, and lower crumb firmness when compared to all other extractions and flour types. These measured bread characteristics were significantly impacted by flour properties, specifically particle size, starch damage, and fiber content ($p < 0.0001$). The commercial flours studied produced breads of low

specific volume, poor crumb properties, and dense textures. These results can assist millers and product developers in advancing the quality of sorghum-based gluten-free foods for the consumers that require them. Further research is necessary to better understand the extent to which particle size, and therefore starch damage, can improve sorghum-based gluten-free breads.

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FOOD ALLERGIES

Introduction

Approximately 1 to 2% of adults and nearly 5% of infants and young children are afflicted with food allergies (Kagan 2003). The United States government reports that each year, around 30,000 individuals require emergency room treatment and at least 150 individuals die because of allergic reactions to food (U.S. Food and Drug Administration 2004). Eight common foods are responsible for more than 90% of food allergies: milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans (Kagan 2003, U.S. Food and Drug Administration 2004).

Mode of Action

While there are several types and causes of adverse reactions from food ingestion, food allergies are specifically categorized as those with immunologic responses. An allergic reaction begins with the ingestion of an allergen. An allergen is typically a protein that is resistant to even the most extreme forms of denaturation, including heat from cooking and processing, stomach acids, and intestinal digestive enzymes (Lehrer and others 2002). Two other immune system components must be involved in order for an allergic reaction to take place. The first is an antibody called immunoglobin E (IgE) that circulates throughout the blood. The other is a specialized cell called a mast cell. While they are typically present in all bodily tissues, mast cells tend to have higher concentrations in areas that are more typical locations of allergic

reactions (i.e. nose, throat, lungs, skin, and gastrointestinal tract) (National Institute of Allergy and Infectious Diseases 2007).

However, before an allergic reaction can occur, a person needs to have been previously exposed, or sensitized, to the allergen. During the first exposure, the allergen stimulates specialized white blood cells, or lymphocytes, to produce the specific IgE antibody for the allergen. The IgE is then released and attaches itself to the surface of the mast cells in the various tissues of the body. When consumed again, the same allergen binds to the specific IgE antibody on the mast cell which prompts the release of chemicals such as histamine (Lehrer and others 2002). These chemicals are the cause of the various symptoms of food allergies.

Symptoms and Treatment

The intricacies involved in digestion affect the timing, location, and specific symptoms of an allergic reaction. In general, the symptoms of a food allergy will occur within a few minutes to an hour of consuming the allergen. According to Kagan (2003), the symptoms can initially be experienced as an itching in the mouth and difficulty swallowing and breathing. Then, once digestion of the food in the stomach and intestines begins, symptoms such as nausea, vomiting, diarrhea, and abdominal pain can start. When an allergen reaches the skin, it can cause hives; if the allergen reaches the lungs, an asthmatic reaction may occur. As the allergen travels through the blood vessels, it can cause lightheadedness, weakness, and anaphylaxis. Anaphylactic reactions can be fatal if not treated quickly (National Institute of Allergy and Infectious Diseases 2007).

There is currently no cure for food allergies. The only treatment is strict avoidance of the allergen-containing food (Kagan 2003, U.S. Food and Drug Administration 2004). However, treatment for allergic reactions with epinephrine is available.

Labeling Regulations

Due to the severity and prevalence of food allergies, the FDA established the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) in August of 2004. This particular act mandated specific labeling for the presence of any of the eight major food allergens by January 1, 2006 (U.S. Food and Drug Administration 2004). Prior to the instatement of this act, food ingredients were required to be labeled with their common or usual name, so as to assist those with food allergies to avoid products of concern (U.S. Food and Drug Administration 2007). However, many allergy-producing foods such as peanuts, eggs, and milk appear in foods that would not typically be associated with them. For example, milk is often present in bakery products, eggs are used in some salad dressings, and peanuts can be used as protein supplements. Likewise, studies have shown that many individuals are unable to correctly identify ingredients that originate from the major food allergens (U.S. Food and Drug Administration 2004). Thus, the FALCPA was aimed at eradicating both consumer confusion and unnecessary allergic reactions.

The FALCPA calls for the labeling of the eight major food allergens in one of two ways. The first option is to identify the common name of the food source from which the allergen originates in parentheses immediately following the name of the allergen in the ingredient statement. However, this is only necessary if the common name of the food

allergen has not been previously used in said ingredient statement. The second option is to include the word "contains" followed by the common name of the food source from which the allergen originates. This language is to be placed immediately after or adjacent to the ingredient statement (U.S. Food and Drug Administration 2004). In its current standings, the FALCPA does not mandate advisory labeling for potential cross-contamination with allergenic substances (U.S. Food and Drug Administration 2008). Additionally, this regulation makes an exception for "highly refined oils," such as peanut oil and soybean oil (Food Allergy and Anaphylaxis Alliance 2002).

CELIAC DISEASE

Introduction

As previously stated, wheat is considered to be one of the eight major allergens that cause more than 90% of allergic reactions from food. However, wheat is also the culprit in another increasingly common condition in today's population: celiac disease. Celiac disease, also known as gluten-sensitive enteropathy, is an autoimmune disorder that affects genetically susceptible individuals. Those affected have adverse reactions upon ingestion of wheat gluten, as well as proteins in related cereals, such as barley, rye, and possibly oats. Portions of these proteins elicit an autoimmune response that causes inflammation of the upper small intestine, thus causing a variety of undesirable symptoms (Alaedini and Green 2005). Studies in both the United States and Europe show the disease affects about 1% of the population (Wieser and Koehler 2008).

Mode of Action

The exact cause of celiac disease is unknown. However, research does indicate that the condition is strongly linked with human leukocyte antigens (HLA) DQ2 and DQ8, as well as other currently unknown non-HLA genes located on chromosome 6 (Wieser and Koehler 2008). These genes seem to be involved in the regulation of the body's primary immune response to gluten protein fractions and to tissue transglutaminase (Celiac Disease Foundation 2008). More specifically, the immune system adversely reacts to the gliadins and glutenins of wheat, secalins of rye, hordeins of barley, and perhaps the avenins in oat (Wieser and Koehler 2008).

Symptoms and Related Conditions

When individuals with celiac disease ingest the aforementioned proteins of concern (simply referred to as "gluten" from here on out), the upper portion of the small intestine is damaged. More specifically, the villi, tiny finger-like projections that absorb nutrients from food, become damaged (Leeds and others 2008). These impaired villi are unable to properly absorb even the most basic nutrients: proteins, carbohydrates, fats, vitamins and minerals. In some cases, even the absorption of water and bile salts is inhibited (Alaedini and Green 2005, Celiac Disease Foundation 2008). Patients with celiac disease exhibit a wide variety of symptoms, ranging from asymptomatic to severe malnutrition. The most common manifestations of celiac disease include abdominal pain and/or distention, chronic diarrhea, steatorrhea (fatty stools), vomiting, weight loss, weakness, and malabsorption (Alaedini and Green 2005, Wieser and Koehler 2008).

The gluten intolerance exhibited in celiac disease is sometimes manifested in the form of dermatitis herpetiformis, a skin disease characterized by blistering and intense itchiness. The lesions are usually symmetrical and are found on the face, elbows,

knees, and buttocks (Alaedini and Green 2005). There is a possibility that patients with dermatitis herpetiformis will have accompanying intestinal damage, but without distinguishable symptoms. This condition is seen in about 10-20% of patients with celiac disease (Leeds and others 2008).

There appears to be a close association between celiac disease and other disorders. In fact, celiac disease is increasingly diagnosed in patients with mostly non-gastrointestinal symptoms including osteoporosis, anemia, and certain reproductive problems (Alaedini and Green 2005). If the malabsorption resulting from celiac disease goes undiagnosed or untreated, there is an increased risk of both nutritional and immune related disorders. Associated conditions include type-1 diabetes, thyroid disease, incidences of some cancers, and neurologic disorders (Alaedini and Green 2005, Wieser and Koehler 2008).

Diagnosis

Recognizing celiac disease can sometimes be difficult, as many of its symptoms are similar to those of other diseases. Celiac disease is often confused with irritable bowel syndrome, Crohn's disease, and diverticulitis. As such, the disease is widely under diagnosed or misdiagnosed. In fact, it is estimated that only 10-15% of those with celiac disease in Europe and North America are currently diagnosed (Wieser and Koehler 2008).

The first step in diagnosing celiac disease is a non-invasive blood test to measure the levels of autoantibodies that are commonly found at higher levels in those with celiac disease: immunoglobulin A (IgA), anti-tissue transglutaminase (tTGA), and IgA anti-endomysium antibodies (AEA) (Alaedini and Green 2005). These results can

be paired with those from both a duodenal biopsy and tissue histology that display damage to the intestinal mucosa (Wieser and Koehler 2008). However, if the patient has been prescribed or has self-prescribed a gluten-free diet in the interim, the intestinal villi begin in heal within weeks, and the biopsy can present a false negative (Leeds and others 2008).

Treatment: Gluten-free Diet

The only available treatment for celiac disease is the strict, lifelong avoidance of gluten. When gluten is removed from the diet, the small intestine will start to heal and overall health should improve (Celiac Disease Foundation 2008, Kupper 2005). A gluten-free diet entails the exclusion of grains such as wheat, barley, and rye. Studies show that oats may be acceptable for patients with celiac disease, and can even improve the nutritional profile of the diet. However, inclusion of oats is not recommended for U.S. consumers due to concerns about potential cross contamination from other grains of concern (Alaedini and Green 2005, Kupper 2005). Traditional substitutes for gluten-containing grains have been rice, tapioca, corn, and potato. However, more nutrient-dense grains, seeds, nut flours, and legumes are being incorporated into the diet to provide an enhanced palatability, variety, and nutritional quality. These include sorghum, millet, tef, quinoa, amaranth, flax seed, and buckwheat (Kupper 2005).

While adherence to a gluten-free diet effectively manages celiac disease, complete avoidance of gluten is not a simple task in an age in which wheat flour is nearly ubiquitous in processed foods. Those with celiac disease should be familiar with the many foods that have hidden and unexpected sources of gluten, including thickened

sauces, soups, pudding, sausages, and soy sauce. In the same way that consumers have difficulty identifying food sources of food allergens, celiac consumers have the same difficulty identifying sources of gluten. While there is no current definition for "gluten-free," foods are, at present, deemed "gluten-free" according to the Codex Standard for Gluten-Free Foods adopted by the Codex Alimentarius Committee on Nutrition and Food for Special Dietary Uses. However, more stringent and specific regulations for defining the claim "gluten-free" were proposed by the FDA in January 2007. Under this proposal, foods that bear the claim "gluten-free" cannot contain prohibited grains, nor ingredients derived from such grains. Additionally, the food must not contain more than 20 parts per million of gluten (Federal Register 2007). Use of the term "gluten-free" will be voluntary. Under regulations set by the European Union, only foods that contain less than 20 parts of gluten per million can utilize the term "gluten-free" on packaging. Foods that have undergone processing to remove most of the gluten that contain less than 100 parts of gluten per million are able to make the claim "very low in gluten" on the packaging. Compliance with this regulation goes into effect January 1, 2012 (Food Standards Agency 2009).

GLUTEN-FREE MARKETPLACE

Aside from the portion of the population that requires a gluten-free diet due to celiac disease or wheat allergies, patients with other ailments can benefit from the adoption of a gluten-free regimen. Following a gluten-free diet is sometimes suggested to patients with Crohn's disease, ulcerative colitis, and irritable bowel syndrome (Engleson and Atwell 2008). Additionally, preliminary studies show some degree of

behavioral improvement in children with autistic spectrum disorders when a gluten-free and/or casein free diet is prescribed (Cromley 2008). While the majority of people on a gluten-free diet are doing so for medical reasons, family members of those with celiac disease may choose to adopt the diet as well to reduce cross-contamination in the home and aid in the ease of preparation of gluten-free foods. There is also an increasing sector that chooses a gluten-free diet because they believe doing so will improve their overall health and well being (Engleson and Atwell 2008).

      With all the individual population segments that require and/or desire gluten-free diets, there are substantial market opportunities for gluten-free foods. According to a March 2007 survey by Mintel, a market research company, 8% of the U.S. population is seeking gluten-free products (Cromley 2008). While some would still consider this a niche market, it was reported by Nielsen Co., which has tracked gluten-free food in U.S. food, drug, and mass (FDM) merchandiser stores (excluding Wal-Mart), that the gluten-free sector increased 20% in the 12-month period ending June 14, 2007 to $1.75 billion. This value is up from $1.46 billion one year ago (A C Nielsen 2007). In 2007, 700 new gluten-free products were launched in the U.S., compared with 214 product launches in 2004, thus indicating an expansion in variety (Cromley 2008, Innova 2007). While sales of gluten-free products via health and natural foods retailers make up 40% of the retail bakery share, these same products are now being sold in larger, more commonplace outlets (Figure 1). Today, Wal-Mart's gluten-free products make up about 5% of the total gluten-free bakery sales (Associated Press 2005, Nielsen 2007). Mintel projects a 15-25% annual growth rate for gluten-free foods over the next few years (Cromley 2008).
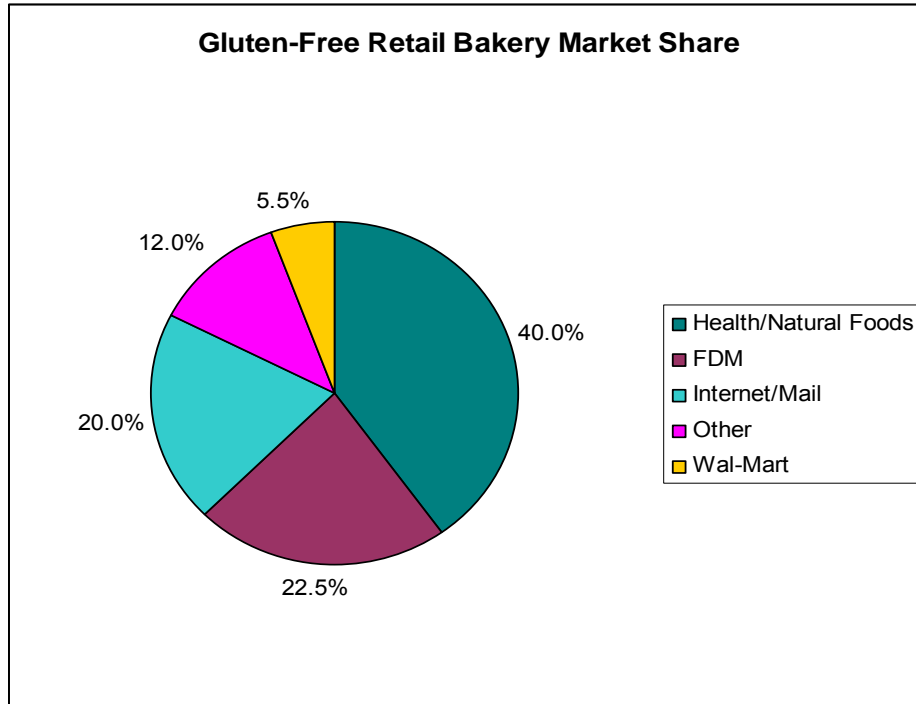
Figure 1. Gluten-Free Retail Bakery Market Share. Adapted from: Nielsen (2007).

SORGHUM

Introduction

With an increasing sector of the population desiring gluten-free foods, there are many opportunities to utilize sorghum, a gluten-free grain. *Sorghum bicolor* (L.) Moench is a cereal in the grasses (Poaceae) family. The grain is native to the tropical areas of Africa, and was first domesticated around 3,000 to 5,000 years ago (U.S. Grains Council 2008b). The genus *Sorghum* was established in 1794, and was then divided into three species: *S. halepense*, *S. propinquum*, and *S. bicolor* (Clayton 1961). Today, the cultivated species, *S. bicolor*, has been further divided into four classifications of sorghum by intended purposes: grain, sweet, broom and grass. Sweet sorghum is used for producing sweetener syrup; broom sorghum is used for making brooms. Grass sorghum's purpose is for feed and forage use, while grain sorghum is mainly used as a

human food source, as well as a raw material for alcoholic beverages, sweets, and glucose production (U.S. Grains Council 2008b).

Sorghum Production

According the to the U.S. Grains Council, sorghum is the third most important cereal crop grown in the United States and the fifth most important cereal crop grown in the world behind wheat, rice, corn, and barley (2008a). While the Food and Agriculture Organization (FAO) reports that sorghum makes up less than 5% of the world grain production, sorghum constitutes the major source of energy and protein for many people in the semi-arid zones of Africa and Asia (Taylor and Dewar 2001). As a continent, Africa is the largest producer of sorghum, with sorghum representing around 70% of the cereals produced in West Africa, 30% in East Africa, and 10% in Southern Africa (Dendy 1995, U.S. Grains Council 2008a). As a nation, the United States is the world's largest producer and exporter of grain sorghum, with approximately 2.5 million hectares grown each year, with India, Nigeria, and Mexico trailing behind (U.S. Grains Council 2008a, 2008b). U.S. sorghum production is concentrated mainly in the central and southern plains states: Kansas, Texas, Nebraska, Oklahoma, and Missouri (U.S. Grains Council 2008a).

The prevalence of sorghum in Africa and areas of the Middle East is mostly due to drought tolerance and the grain's ability to sustain considerable degrees of water logging. Thus, the ease of growing lends itself as a major food crop in these areas. However, the main function of grain sorghum is as animal feed, especially in the U.S., Mexico, South America, and Australia (U.S. Grains Council 2008a). Because of nutritional similarities to corn, sorghum is often used to feed beef and dairy cattle, laying

hens, pigs, and in pet foods. Aside from food and feed use, approximately 12% of U.S. sorghum production is utilized in the production of ethanol and its various co-products (U.S. Grains Council 2008a).

Structure and Appearance

To the untrained eye, sorghum plants are often mistaken for corn plants. The height of the plant ranges from 60cm high to nearly 460cm high. On the stalk grows the long, wide leaves, topped with the seed head. A mature sorghum plant has a seed head of about 25 to 36 cm tall containing small round seeds (U.S. Grains Council 2008b) (Figure 2). According to Rooney and Clark, the sorghum kernel is a flattened sphere around 4.0mm long, 3.5mm wide, and 2.5mm thick (1968). Sorghum kernels vary in size from 1.0 to 3.0 grams per 100 kernels (Wall and Blessin 1969). The grains range in color from white to shades of yellow, red, and brown. The original color of sorghum was either purple or red with a red seed coat. However, the color and taste were considered inappropriate for use as a food crop, and thus, efforts were made in breeding to make improvements. The result was the development of a white sorghum with a white seed coat, a champagne colored body, and a wheat colored head (U.S. Grains Council 2008b).
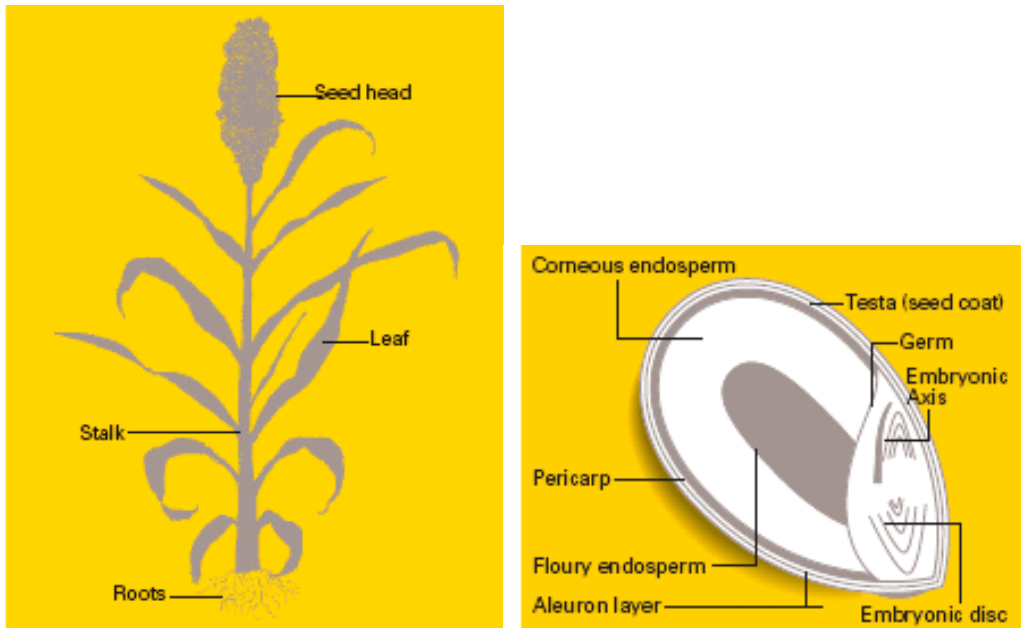
Figure 2. Sorghum plant structure (left) and sections of a sorghum seed (right). Source: U.S. Grains Council (2008b).

With the exception of its much smaller size and slightly oval shape, the structure of the sorghum kernel, or caryopsis, is very similar to corn. The kernel is composed of three main parts: the outer covering (pericarp), the embryo (germ), and the storage tissue (endosperm). Each of these parts can be further subdivided. The pericarp has three distinct layers. The outer layer is the epicarp, typically containing pigments and/or a waxy cuticle (Wall and Blessin 1969). The middle layer, or mesocarp, is made up of large, thin-walled cells that contain several layers of starch granules; this feature is unique to sorghum. The innermost layer of the pericarp, or endocarp, is composed of cross and tube cells (Rooney and Clark 1968). While absent in most, some varieties of sorghum contain what is referred to as the testa, or a layer of highly pigmented cells beneath the pericarp. Contained in these cells are condensed tannins, or proanthocyanins, which provide significant agronomic advantages such as protection from bird, insect, and fungal predation (Taylor and Dewar 2001, Wall and Blessin 1969).

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The germ of the sorghum kernel is located at the base of the seed, and is tightly embedded within the kernel, causing difficulty for removal during dry and wet milling (Rooney and Clark 1968; Wall and Blessin 1969). Corn and sorghum both have a large germ fraction relative to the size of the endosperm fraction. As a result, sorghum tends to have higher oil content (3.4%) in comparison to wheat (2.2%). Thus, sorghum flour typically has a higher fat content, leading to issues with oxidative rancidity (Taylor and Dewar 2001).

The centrally located sorghum endosperm is made up of an outer aleurone layer, a peripheral region of horny, or corneous, endosperm, and an inner layer of soft, floury endosperm. The aleurone layer is located directly beneath the pericarp, and consists of cells that are relatively high in oil and protein (Wall and Blessin 1969). The horny endosperm fraction is located more to the outside of the kernel and is characterized by small starch granules that are contained within a thick protein matrix. The remainder of the endosperm consists of larger starch granules that make up the floury endosperm (Rooney and Clark 1968). Because the kernel contains both hard and soft endosperm, milling sorghum can be challenging.

Composition

With the exception of a lower oil content, the composition of grain sorghum is quite similar to corn. According to Wall and Blessin, the endosperm makes up 82% of the grain; the germ, 10%; and the pericarp 8% (1969). Rooney and Clark compiled the compositions of and within the caryopsis; these results are shown in Table 1 (1968, Waniska and Rooney 2000). The whole grain tends to be relatively low in fiber (2.7%), contains 7 to 15% protein, and up to 75% starch. As previously mentioned, the germ is

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a rich source of oil and protein (28% and 18% respectively), and the majority of the endosperm is composed of starch and protein (Rooney and Clark 1968, Wall and Blessin 1969, Waniska and Rooney 2000). The composition of sorghum grain may fluctuate between different sources due to the hybridization, environmental conditions, and crop management (Wall and Blessin 1969).

Table 1. Chemical Composition (%) and Anatomical Tissues of Sorghum		Caryopsis	Endosperm	Germ	Pericarp
Caryopsis		100	84.2	9.4	6.5
	Range		81.7-86.5	8.0-10.9	4.3-8.7
Protein		11.3	10.5	18.4	6
	Range	7.3-15.6	8.7-13.0	17.8-19.2	5.2-7.6
	Distribution	100	80.9	14.9	4
Fiber		2.7			
	Range	1.2-6.6			
	Distribution	100			
Lipid		3.4	0.6	28.1	4.9
	Range	0.5-5.2	0.4-0.8	26.9-30.6	3.7-6.0
	Distribution	100	13.2	76.2	10.6
Ash		1.7	0.4	10.4	2
	Range	1.1-2.5	0.3-0.4		
	Distribution	100	20.6	68.6	10.8
Starch		71.8	82.5	13.4	34.6
	Range	55.6-75.2	81.3-83.0		
	Distribution	100	94.4	1.8	3.8

Source: **Data from Rooney and Clark (1968).**

Starch

Sorghum and corn starches have similar properties. The granules are the same shape, but sorghum starch granules are slightly larger. There are minor differences in the rheological characteristics of the starch, including swelling capacity and paste viscosity (Rooney and Clark 1968). The gelatinization temperature of sorghum starch ranges from 71°C to 80°C, which is higher than corn, which gelatinizes from 62 $^{\circ}$C to 72 $^{\circ}$C. Therefore, sorghum requires a longer cooking time and more thermal energy during processing (Waniska and Rooney 2000). Rooney and Clark report that most sorghum starches contain 20 to 30% amylose and 70 to 80% amylopectin; however, waxy varieties (less than 5% amylose) have been developed (Rooney and Clark 1968).

Protein

The majority of sorghum proteins are found within the endosperm of the kernel, distributed within both protein bodies and the endosperm's protein matrix. The major sorghum protein fractions are prolamins and glutelins, with prolamins predominating. The prolamin in sorghum is kafirin—a protein similar to maize zein in molecular weight, structure, solubility, and amino acid composition (Seckinger and Wolf 1973, Da Silva and Taylor 2005). Kafirin is found to predominate in three forms: α-, β-, and γ-kafirin, with α-kafirin being the major form (Musigakun and Thongngam 2007). These sorghum prolamins are located within the protein bodies in the starchy endosperm (Taylor and others 1984). Like many cereals, the limiting amino acid in sorghum is lysine (Waniska and Rooney 2000). Sorghum proteins lack the ability to form a gas-holding network in a viscoelastic dough. Therefore, sorghum's use in baked products has been limited thus far.

Fiber

Sorghum contains 6.5 to 7.9% fiber, of which 86.2% is insoluble (Waniska and Rooney 2000). This fiber functions as a structural and protective feature. In cereal grains, dietary fiber (made up of soluble and insoluble fractions) is generally located in the pericarp. As such, the fiber composition in sorghum products, such as flour, depends on the degree of pericarp removal or extraction level. The insoluble fiber components in sorghum are rich in glucuronoarabinoxylans. In comparison, barley is rich in soluble beta-glucans, while wheat has both soluble and insoluble arabinoxylans (Taylor and Dewar 2001). These differences in fiber composition may have a significant impact when sorghum is used in value-added products.

Lipids

As previously mentioned, the majority of lipids found in sorghum are located in the germ. The fatty acid composition of these lipids is quite similar to those found in corn oil. The sorghum oil is primarily made up of the following fatty acids, in order of decreasing concentration: linoleic, oleic, palmitic, stearic, myristic, and hexadecanoic (Rooney and Clark 1968, Waniska and Rooney 2000).

Traditional Sorghum Food Products

While sorghum has traditionally been used in the United States as animal feed, nearly 40% of the global sorghum crop is used for human consumption (Waniska and Rooney 2000). However, the growers in the U.S. are increasing their interest in producing human food products from sorghum due to the development of the aforementioned white sorghum. White sorghum has a neutral flavor and color profile, thus allowing this type of grain to be more easily incorporated into new food products.

There is a wide array of traditional sorghum products that currently exist in areas where sorghum production is abundant, namely Africa and India. While there are many regional variations, the categories of these products remain fairly consistent. Table 2 shows groupings of these traditional products compiled by Waniska and Rooney (2000), and modified by Schober and others (2006). These categories include breads (both fermented and unfermented), porridges, rice-like products, and beer. Sorghum bread and baked goods will be discussed more in detail later.

Table 2. Examples of Traditional Sorghum Products		
Food	**Example**	**Region of Origin**
Unfermented Bread	*chapati*	India, East Africa
Fermented Bread	*dosa*	Southern India
Stiff Porridge	*ugali*	Southern/East Africa
Thin Porridge	*uji*	East Africa
Steamed Products	*couscous*	West Africa
Alcoholic Beverages	*dolo*	West Africa
Sour/Opaque Beer	*busaa*	East Africa

Adapted from Waniska and Rooney (2000), Schober and others (2006).

While many cultural variations exist, a staple food made from sorghum is porridge. Porridges are prepared by cooking either fermented or unfermented slurries of sorghum flour in boiling water. They are typically either thick or thin, with the differences in viscosity caused by flour concentration, pH of the cooking water, flour particle size, and endosperm hardness (Anglani 1998, Taylor and Dewar 2001). Flavor of the porridges is determined by the extent of fermentation treatment on the sorghum flour. These are included in meals at all times of the day, as well as weaning foods for small children.

Whole or decorticated sorghum is often consumed as a rice-like product in several African countries: in Sudan, "*pearl dura*"; in Kenya, "*supa mtama*"; and South Africa, "corn rice" (Taylor and Dewar 2001). The main drawback to these products is their long cooking time. For example, South African corn rice must be rinsed, pre-cooked for 10 to 15 minutes, rinsed again, and then cooked for another 25-35 minutes (Perten 1983). Another rice-type product made with sorghum is couscous. While couscous is traditionally known in the U.S. as a short grain pasta product made from wheat semolina, sorghum couscous is a steamed, agglomerated food made from the flour of decorticated sorghum (Anglani 1998). Like porridges, couscous is served for breakfast, lunch or dinner with either buttermilk or more savory items such as fish or vegetables.

Beer and alcoholic beverages have been made from sorghum throughout southern, central, and eastern Africa at least since the 1980s. These differ from clear, European-style beers, in that they are opaque and viscous, due to the suspension of cereal, starch, and yeast particles. They are also lower in alcohol content (3% w/w), and are not typically pasteurized. These beverages are characterized by a sour, lactic acid flavor, provided either by lactic acid fermentation or by the addition of commercially produced lactic acid (Taylor and Dewar 2001).

SORGHUM MILLING

Milling Objectives

Dry milling of grain sorghum is a small, but expanding segment of the milling industry. Limited publications exist on sorghum milling techniques, and processes developed for individual sorghum milling operations remain proprietary. However, as research regarding sorghum utilization continues to expand, milling techniques are being developed that take advantage of the unique characteristics of the sorghum kernel.

The objective of any flour milling operation is twofold: the first is to reduce the kernel into finer particles; the second is to achieve an efficient separation of the kernel entities. More specifically, it is desirable to produce the cleanest possible separation of endosperm, germ, and bran, while recovering a maximum yield of endosperm. Depending on the objective, either clean endosperm grits or flour are the desired end-products (Hahn 1969). For flour production—the focus of this review—there are two general approaches to milling. The first involves the removal of the germ and the outer layers of the kernel (including pericarp, seed coat, aleurone, and nucellus—referred to

as "bran"), collectively called "degermination." This step is followed by subsequent reduction of the remaining endosperm. This method is utilized in maize milling. Alternatively, the kernel can first be broken open, allowing the endosperm to be scraped from the bran layer. This method is primarily utilized for wheat and rye milling. With either method, the endosperm fraction should have as little bran and germ contamination as possible, as these products discolor the flour and affect the shelf-life of the product (Taylor and Dewar 2001). Additionally, for both methods, a product composed of overlapping kernel fractions is obtained, designated as "shorts" or "fines." Included in this fraction are portions of all milling streams, which due to composition, color, or most importantly, particle size, cannot be combined into the flour or bran fractions. To do so would sacrifice product quality (Hahn 1969).

Sorghum Milling

Structural Challenges

The sorghum kernel has several unique characteristics that provide challenges and opportunities during milling. Several decades ago, a review of the then-current state of sorghum milling technology was published (Hahn 1969), and several observations are still pertinent at present. It was noted that the bran of sorghum is more friable (fragile) than wheat bran, and as such, it tends to fracture more easily into small pieces that contaminate the endosperm fraction, rendering the flour, in milling terminology, "specky." Additionally, due to the small, slightly flattened, round shape of the sorghum kernel, there is difficulty in making clean separation of the components, including removal of the germ. As a result, the sorghum endosperm becomes fractured during the degermination process, and bran contamination occurs. Tempering, a process in which

water is added to the grain and allowed to equilibrate throughout the kernel over a period of time, aids in freeing the germ while keeping the endosperm intact. This process has several effects on the kernel, including causing the bran to become tough and rubbery, making the endosperm soft and friable, and resulting in a slight swelling of the germ. All of these effects from tempering allow the germ to pull away from the endosperm more easily (Hahn 1969). If, during the same process, the endosperm remains intact, the germ and endosperm can easily be separated through sieving (Taylor and Dewar 2001). Tempering does in fact assist with germ removal, but this method is not always successful in keeping the endosperm intact. As a result, the method of decortication has been developed to remove the outer portion of the kernel by abrasion with minimum cracking of the endosperm.

Decortication

Decortication is an alternative method of removing the bran and germ of the sorghum grain, effectively abrading off the outer layers of the kernel. The process is also known as pearling or dehulling, with the latter term considered a misnomer, as the sorghum caryopsis is "naked," or without a hull. It is actually the pericarp and germ which are being removed through this process (Taylor and Dewar 2001). In tannin-containing varieties of sorghum, decortication is the most common and beneficial approach to milling, as this step aids in the removal of the testa, and thus reduces the tannin content of the grain and flour (Serna-Saldivar and Rooney 1995).

The most common type of decortication equipment is the Prairie Research Laboratory (PRL) dehuller, based upon the design of a barley thresher or pearler. The machine consists of a horizontal cylinder with perforations, containing 6 to 13 evenly

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spaced Caborundum disks that rotate clockwise against the grains at approximately 2000 rpm (Schmidt 1992). As a result, the bran and germ are progressively abraded off, and are subsequently separated by either sifting or aspiration. This process can be repeated as necessary until the desired removal of tissue is achieved (Hahn 1969). While the intention of the process is to limit endosperm fracture, excessive breakage is still an issue. Additionally, not all of the germ gets removed, and thus the endosperm meal still has an unacceptably high fat content, between 2 and 4 percent (Taylor and Dewar 2001).

These issues led researchers to carefully study the traditional hand-pounding milling technique that has been used for sorghum in developing countries. In this mortar-pestle process, the pestle generates interactive forces between the grains. With the addition of water (tempering), large flakes of the grain are removed, rather than the fine bran flour that is produced in abrasive decortication. By applying this principle of attrition, United Milling Systems developed a decorticator that uses a steel rotor to squeeze the tempered sorghum kernels against both one another and a cylindrical screen. The bran and endosperm fragments are discharged through the screen by high pressure air current. If the grain is properly tempered, this type of decorticator allows a near-complete and clean removal of the germ from the endosperm (Munck and others 1982, Taylor and Dewar 2001).

Dry Roller Milling

More recently, research efforts have returned focus to applying wheat roller milling technologies and equipment to the milling of sorghum. While this technique is able to produce a somewhat more bran- and germ-free product, the main advantage to

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roller milling is the incorporation of "reduction" rolls, and as such, the production of finer sorghum flour. For this process, the top pair of rollers are coarsely corrugated "break" rolls, and the second pair are finer corrugated break rolls. If present, the third pair of rolls are smooth reduction rolls. Studies have shown that with moderate pre-conditioning (tempering to 16% moisture), roller milling can consistently produce sorghum flour of higher extraction and lower ash and fat levels compared to sorghum flour produced by decortication and subsequent hammer milling (Gomez 1993, Hammond 1996).

Yield of the flour produced by the break rolls ranges from 10 to 15% and contains most of the floury endosperm, which is low in protein. The flour produced from the reduction roll contains a greater amount of bran and germ particles, and is "specky" and higher in oil content. Both the appearance and properties of the straight-grade flour (combination of break and reduction flours) can be considerably improved by milling to a lower extraction rate (Hahn 1969).

Semi-wet Roller Milling

Some researchers regard separation of sorghum grain constituents in dry roller milling as poor. Cecil (1992) described a semi-wet roller milling process for sorghum that resulted in more effective separation of the bran and germ. Research showed that with the addition of 20% water to the grain and conditioning at 60°C for 6 hours, sorghum could be efficiently milled on standard wheat roller mills. This water addition is far more than is used in dry milling, but considerably less than is used in wet milling, thus named semi-wet milling. This process was reported to yield "high quality, fine flour" (Cecil 1992). Additionally, this process was able to effectively reduce tannin content in

the flour when red or broom-corn varieties of sorghum were milled. However, the disadvantages of this method may outweigh the benefits. Issues include microbiological growth inside the mill, poor flour yield, necessary removal of residual moisture in flour and byproducts, and screen blockage due to poor flow characteristics (Cecil 1992, Taylor and Dewar 2001).

GLUTEN-FREE SORGHUM BREAD

Wheat Bread

The most ubiquitous form of leavened pan bread is made with wheat flour. When mixed with water, wheat flour forms a cohesive and visco-elastic dough. This matrix is able to hold gas cells nucleated by yeast fermentation and/or chemical leavening. Upon baking, this dough sets to form a sponge-like structure. Wheat is the only cereal that is able to form a product with such gas-holding properties, and it is generally accepted that the gluten proteins of wheat are responsible (Hoseney 1994). More specifically, research has shown that the high molecular weight glutenins of wheat impart the elasticity that is responsible for the visco-elastic dough (Khatkar and Schofield 1997). As previously discussed, sorghum does not contain such structure-forming proteins. Thus, producing high-quality gluten-free sorghum bread that is acceptable to consumers is a challenge.

Sorghum-Wheat Composite Breads

A well-established use for sorghum in leavened baked products has been in sorghum-wheat composite breads. Composite breads are made from a blend of two or more flours from different sources, with the most common approach using wheat flour

plus one or more other flour. In 1964, The Food and Agriculture Organization (FAO) initiated the "Composite Flour Program" to find new uses for raw materials other than wheat for the production of bread, cookies, pasta, and other flour based foods (Taylor and Dewar 2001). Most of the research was carried out for the benefit of developing countries that were having difficulties importing wheat due to the high cost. As a result, numerous studies were undertaken in using sorghum as an ingredient in conventional wheat bread. According to Dendy (1992), the world-wide research on sorghum-wheat composites showed that up to 30% sorghum flour could be used in conjunction with a wheat flour of "reasonable strength" to make quality bread. Additionally, sorghum-wheat composite breads have been shown to achieve higher loaf volumes, enabling wheat flours with poor quality gluten to be used to make bread (Pringle and others 1969). A study was done by Perten and others (1983) comparing sorghum bread to bread made with wheat flour. The volume of bread made with sorghum alone was smaller than that of wheat flour bread, but the sorghum bread was preferred by many consumers. Despite this, the authors suggested that sorghum flour was not considered a bread-making flour, as it was unable to produce bread with an acceptable volume. They concluded that bread made with 30% sorghum flour and 70% wheat flour of 72% extraction rate was tolerable, and was evaluated by consumers as "good to excellent" by an acceptability panel (Perten and others 1983). While these composite breads provide a use for sorghum, especially in countries where sorghum is the main commodity over wheat, these breads are not gluten-free, and thus, cannot be consumed by those with celiac disease or wheat allergies.

Wheat-Free Sorghum Breads

Compared with the abundant number of studies dealing with sorghum-wheat composite breads, very little research has been undertaken to address the issue of gluten-free sorghum breads that resemble wheat pan breads in appearance and acceptability. Such breads would be suitable for those on a gluten-free diet, as well as providing cost savings and a possible replacement for wheat breads in developing countries where wheat is not the predominately native cereal. Because the aforementioned gluten proteins provide the means for a gas holding, structure forming network, the development of gluten-free breads must enable the use of different technologies.

Batter Viscosity

Hart and others (1970) were some of the earlier researchers to develop basic formulas for non-wheat breads using sorghum flour. It was observed that soft batters (100-150% water based on flour weight), rather than traditional doughs, were necessary to achieve adequate volume and rise. In contrast, firmer "doughs" lacked elasticity, were brittle, and rose insufficiently. Similarly, Schober and others (2005) found that an increase in water levels in order to create a low viscosity batter system improved bread volume. This seems to signify that greater dilution of suspended particles (e.g. bran, endosperm particles) is desirable for the final product (Schober and others 2005). While these batters are intentionally formulated to have low viscosities, it has also been observed that the lack of gluten in sorghum causes the batter to have a more fluid consistency (Cauvain 1998). As a result, gas holding is more difficult, and the result is loaves with low baked volumes. Thus, the use of various functional ingredients must be

enacted to provide a means for gas occlusion and stabilization during baking (Cauvain 1998, Satin 1988).

Hydrocolloids

After coming up with a general formula in their initial studies, Hart and others (1970) then tested the effect of various gums and hydrocolloids. It was found that certain methylcellulose ingredients at usage rates of approximately 4% acted as thickening agents and increased gas retention, prevented loaf collapse, and gave overall larger loaf volumes. Preliminary work from the same study showed that the use of hydroxypropyl methylcellulose (HPMC) improved the quality of fresh bread and delayed staling (as reported in Schober and others 2007). Guarda and others (2004) and Collar and others (2001) reported similar effects in wheat bread, with the results attributed to the water retention ability of HPMC, and its tendency to bind to starch. These actions potentially inhibit amylopectin retrogradation, which is the suspected cause of staling (reported in Schober and others 2007). Schober and others (2005) utilized xanthan gum in sorghum bread formulations. While the addition of xanthan gum was shown to decrease loaf volume, breads produced with xanthan gum did have the benefit of reduced crust cracking. In contrast, Satin (1988) found that the addition of xanthan gum generally produced acceptable sorghum breads, but that the technique for addition was critical for success. The suggestion was made for xanthan gum to be pre-hydrated in water before adding to the batter in order to improve bread quality.

The improving effect of hydrocolloids on sorghum batters align with the findings of Gan and others (1995) for wheat doughs, even though gluten-free batters lack an aggregated protein network. Typically, gas cells in fermenting batters are surrounded by

liquid films which get stabilized by the addition of the above discussed hydrocolloid ingredients. The effect is also seen with the inclusion of other surfactants, polar lipids, soluble proteins, and soluble pentosans. (Gan and others 1995, Schober and others 2005).

Starch

The inclusion of pure starches (either native or pre-gelatinized) has also been shown to improve quality of gluten-free sorghum breads. Studies done by Olatunji and others (1991, 1992) achieved favorable results using a 70:20:10 blend of decorticated sorghum flour, gelatinized cassava starch, and raw cassava starch, respectively. The batter had 100-110% total water (flour/starch basis). In the same work, research showed that gluten-free sorghum bread could still be produced without any type of additional hydrocolloid, stabilizer, or pre-gelatinized starch when approximately 30% raw starch was added to the sorghum flour (Olatunji and others 1992). Hugo and others (1997) built on Olatunji's findings and used the aforementioned base formula, but added shortening, which resulted in increased volume and decreased staling. Taylor and others (2006) found that the use of starches in combination with methylcellulose improved oven rise and crumb structure. Various starches were used (modified sorghum, waxy sorghum, corn, cassava, arrowroot, potato) and similar results were produced.

There are several explanations of starch's role in improving gluten-free sorghum bread. Gan and others (2005) suggest a dilution effect, in that endosperm and bran particles in sorghum flour become diluted by the added starch. These particles would most likely disturb the homogeneity of the starch gel and obstruct uniform gas cell

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formation. Schober and others (2005) suspected that gluten-free breads from whole grain flours would have lower volumes than pure starch breads for the same reasons. Additionally, the addition of pure starch would affect the overall starch gelatinization of the batter. Sorghum's high starch gelatinization temperature appears to be a factor that impedes quality of gluten-free sorghum breads. Therefore, the addition of pure starch would allow the batter to gelatinize faster and to completion. More rapid gelatinization would result in an earlier increase in batter viscosity, and thus a more even crumb consistency. By contrast, low batter viscosity during the baking process would lead to collapsing of the loaf (Schober and others 2007). Schober and others (2005) hypothesized that pre-gelatinized starch might have the ability to trap air bubbles in sorghum breads due its hydrocolloid properties.

Other Additives

Beyond starches and hydrocolloids, attempts to use other quality-improving ingredients have been made. Along with the studies on the effects of various hydrocolloids, Hart and others (1970) also investigated the effects of enzymes, emulsifiers and shortenings on gluten-free sorghum bread. Alpha-amylase, proteases, and emulsifiers were found to weaken the crumb structure. However, shortenings combined with methylcellulose softened the loaves. Schober and others (2006) researched the effect of skim milk powder in gluten-free sorghum bread. While the ingredient decreased loaf volume, the addition of the skim milk powder did improve crust browning which assists in increasing its consumer acceptability.

Despite the success of the aforementioned formulations, the studies of Olatunji and others (1991, 1992), Hugo and others (1997) and Schober and others (2005) have

shown that hydrocolloids or other complex ingredients are not required to produce leavened sorghum bread. While select additives may be used to make improvements in quality and shelf life extension, a simple recipe would assist in lowering costs for developing nations (Schober and others 2006).

Hybrid

Hugo and others (1997) looked at three sorghum cultivars of varying endosperm types (normal, hetero-waxy, and waxy) for use in gluten-free sorghum bread. The formula was based on the sorghum and cassava blend used by Olatunji (1992). Normal sorghum produced the most acceptable bread. However, the waxy endosperm sorghum resulted in "unacceptable bread having a large hole and a pudding like crumb." The conclusion was made that amylose content has a key functional effect in the production of such a bread system (Hugo and others 1997).

Schober and others (2005) examined the topic of sorghum cultivar by investigating nine selected sorghum hybrids, as well as one commercial sorghum flour. A simple formula was used, also based on the one used by Olatunji (1992). It appeared that bread volume and height were not significantly affected by hybrid. However, significant differences were observed in the crumb grain, including both size and number of cells, as well as texture. While the milling technique for each flour was standardized, each flour had varying levels of starch damage. It was determined that higher kernel hardness resulted in greater starch damage, and that flours with higher starch damage resulted in bread with a coarser crumb structure. This can be attributed to damaged starch's susceptibility to degradation by amylases (Schober and others 2005, 2006).

Starch Damage

As illustrated by the work of Schober and others (2005), starch damage of sorghum flour plays an important role in producing acceptable gluten-free sorghum bread. It was briefly mentioned that the differences in crumb grain from starch damage were most likely due to the action of amylases. More specifically, damaged starch is easily degraded by endogenous amylases, which results in a higher amount of fermentable sugars. This, in turn, creates a weaker starch gel (Schober and others 2005). Munck (1995) suggested that the vitreous endosperm of sorghum creates the variety of crumb textures described by Schober and others (2005). There is a postulation that a kafirin complex encapsulates the starch granules and becomes highly cross-linked and hydrophobic. As a result, water uptake by the starch granules is slowed, and gelatinization is delayed (Chandrashekar and Kirleis 1988).

Difficulties in studying the effect of damaged starch on end-product quality are centered around differences in water absorption. In wheat dough research, a Brabender farinograph is typically used to determine optimum water absorption. However, with this not being possible in sorghum applications due to the lack of gluten network formation, alternate measures must be taken. In the work of Schober and others (2005), an extrusion cell and a computerized texture analyzer were used to standardize batter consistency to a constant viscosity. This was based on the work of Sanchez and others (2002).

EFFECT OF PARTICLE SIZE ON PRODUCT CHARACTERISTICS

Introduction

As previously discussed, gluten-free bread systems must employ different technologies to make up for the lack of a protein network that holds gas and creates structure. Several methods of doing so were mentioned, including the use of various ingredients that assist in creating cohesiveness and holding particles together. Hoseney (1994) noted that for wheat, finer flour particle size—with the correct absorption—can create more cohesion in most baking systems. Unfortunately, research on the effects of sorghum flour particle size on gluten-free bread systems appears to be non-existent. As a result, studies on the effect of wheat flour particle size are being utilized to make inferences and hypotheses as to how sorghum flour may perform.

Layer-Cake Quality

Studies dating back as early as 1919 (LeClerc and others) have investigated the effects of wheat flour granulation and particle size on baking quality. Shellenberger and others (1950) compiled the most prominent studies done on the topic. Amongst other conclusions, it was made evident in the studies that layer cake quality improved with a decrease in particle size when chlorinated soft wheat flour was used. These results were corroborated by the studies of Miller and others (1967), Rees (1971), Yamazaki and Donelson (1972), and Chaudhary and others (1981). All reports suggested a highly significant correlation between particle size and cake volume. In addition to noting a relationship, Chaudhary and others (1981) also determined that endosperm fracturing properties inherent of each wheat variety affected particle size, and as a result, influenced cake quality. The study included the use of wheat that represented the major classes and subclasses grown in the United States. Each wheat class was milled to both straight and patent grades, and then subsequently pin milled to produce smaller

particle size fractions. Among these flours it was noted that in hard red spring wheat, flour particles were significantly reduced in size upon pin milling. However, in contrast, soft red winter wheat did not respond in a similar manner. While the overall conclusion of the study determined that particle size contributed to differences in cake volume, it was also suggested that the 'component governing flour granularity' was also responsible for the cake quality (Chaudhary and others 1981).

Flour Tortillas

While studies on the effect of flour particle size on cake volume relate most to the development of a batter-based bread system, other researchers have reported significant findings on the effect of wheat flour particle size on additional food products. Wang and Flores (2000) examined the effect of particle size on textural properties of wheat flour tortillas. Hard red winter (HRW), hard white winter (HWW), and soft red winter (SRW) wheat varieties were milled and sieved into different particle size fractions. For the particular textural analysis, distance of rupture was measured, with an increase in rupture distance being indicative of a greater force. A greater force signifies greater stretchability, a desired characteristic in wheat flour tortillas. It was found that the finest fractions of all varieties (<38um, 38-53um) of flour yielded tortillas with shorter rupture distances, and undesirable foldability. Tortillas made from the "medium" fractions of HRW and HWW flour (53-75um) had longer rupture distances and superior foldability. By using size exclusion-high performance liquid chromatography (SE-HPLC), the distributions of low molecular weight (LMW) and high molecular weight (HMW) proteins were analyzed, showing differences among varieties. The concentration of LMW proteins was negatively correlated with stretchability. Since the flour fractions

were produced by sieving, this provides a possible explanation for the poor performance of the finest flour fractions. However, particle size of the flours was still the major factor affecting tortilla texture. In contrast, Mao and Flores (2001) found that flour with lower starch damage and coarser particle size produced tortillas with higher stretchability.

Noodles

Hatcher and others (2002) investigated the effect of particle size on quality of white salted noodles. The flours produced were of varying particle size (132-193um, 100-132um, 85-110um) from Canadian Western Red Spring farina. When water absorption was adjusted for optimum handling, water uptake during cooking was highest for fine particle size flour. Instrumental texture measurements also indicated superior cooking quality when particle size was finer, concluding that flour particle size was a primary quality determinant of white salted noodle properties (Hatcher and others 2002).

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CHAPTER 2:

Effect of Sorghum Flour Composition and Particle Size on Quality of Gluten-Free Bread

ABSTRACT

In order to improve the quality of products available for consumers who require a gluten-free diet, this study examined the effects of sorghum flour composition and particle size on functionality in gluten-free batter bread. White, food-grade sorghum was milled to flour of varying extraction rates (60%, 80%, 100%), and was subsequently pin-milled at different speeds (no pin-milling, low- speed, and high-speed) to create flours of both variable composition and particle size. Two commercially-milled sorghum flour samples (AF and TV) were included in the study and subjected to the same pin-milling treatments. Characterization of each flour included measurements of flour composition, total starch content, particle size distribution, damaged starch, and water absorption. Bread characterization included measurement of specific volume, crumb properties, and crumb firmness through the use of digital imaging and texture profile analysis.

Significant differences were found ($p < 0.05$) in the composition of sorghum flours of varying extraction rate, most notably for fiber and total starch contents. Flour particle size and starch damage were significantly impacted by extraction rate and speed of pin milling ($p < 0.05$). With the exception of the commercial flour samples, water absorption increased significantly with increasing extraction rate and speed of pin-milling speed ($p < 0.05$).

Within all treatments, breads produced from 60% extraction flour had significantly higher specific volumes, better crumb properties, and lower crumb firmness when compared to all other extractions and flour types. These measured bread characteristics were significantly impacted by flour properties, specifically particle size, starch damage, and fiber content ($p < 0.0001$). The commercial flours studied produced

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breads of low specific volume, poor crumb properties, and dense textures. These results can assist millers and product developers in advancing the quality of sorghum-based gluten-free foods for the consumers that require them. Further research is necessary to better understand the extent to which particle size, and therefore starch damage, can improve sorghum-based gluten-free breads.

INTRODUCTION

Celiac disease is an autoimmune disorder that affects genetically susceptible individuals. It is caused by the ingestion of wheat gluten, as well as proteins in related cereals, such as barley, rye, and possibly oats. Portions of these proteins elicit an autoimmune response that causes inflammation of the upper small intestine, thus causing a variety of undesirable symptoms (Alaedini and Green 2005). Studies in both the United States and Europe show the disease affects about 1% of the population (Wieser and Koehler 2008). The only effective and available treatment is the lifelong avoidance of gluten-containing foods. According to Mintel, 8% of the U.S. population is seeking gluten-free foods, and there is a projected 15-25% annual growth rate for these products over the next few years (Cromley 2008).

Sorghum is widely available in the U.S., being the 3rd most important cereal crop grown with 2.5 million hectares grown each year (U.S. Grains Council 2008a). As sorghum does not contain the gluten proteins harmful to celiac patients, it is an appropriate grain for use in gluten-free products for human consumption.

It is widely accepted that gluten proteins are responsible for the gas-holding matrix that sets the structure in wheat bread (Hoseney 1994). Without these structure-forming proteins, it is a challenge to produce high-quality gluten-free bread that is acceptable to consumers. While there are a handful of commercially available gluten-free breads, these products have an undesirable firm texture, large crumb structure, bland taste, and poor shelf-life.

Studies dating as far back as the 1920s document the effects of wheat flour composition and particle size on end-product quality (LeClerc and others 1919).

However, such studies have not been carried out for the purposes of improving quality of sorghum products, and specifically, gluten-free sorghum bread. At present, it has been observed that commercially available sorghum flour is milled at a very high extraction rate with no particular quality specifications regarding particle size.

Based on the documented and well-understood effects of wheat flour properties on product quality, it is hypothesized that particle size and composition of sorghum flour will affect its functionality in a gluten-free bread system. In testing this research hypothesis, the main objective is to provide information that will assist millers in producing a more value-added sorghum flour that can be successfully used in a variety of applications, as well as to enable product developers to produce higher-quality gluten-free products for consumers that require them. Overall, fulfillment of these objectives will most benefit consumers of gluten-free products.

MATERIALS AND METHODS

SORGHUM KERNEL CHARACTERIZATION

Single Kernel Characterization System

While the single kernel characterization system (SKCS) is widely recognized in the wheat industry as the standard for prediction of end-product quality, Pedersen and others (1996) and Bean and others (2006) have utilized SKCS for the evaluation of sorghum grain attributes. A sample of white, food grade sorghum of Fontanelle variety D-1000-7 was prepared for testing by cleaning on a dockage tester (Carter-Day Company, Minneapolis, MN) using the Federal Grain Inspection Service (FGIS) wheat

cleaning configuration. Air control was set to a level 4, and feed control was set to a level 6. No sieve was placed in the top carriage, and a No. 2 sieve was placed in both the middle and bottom carriages. This procedure assisted in removing broken kernels, erroneous seeds, and other foreign material. The cleaned sorghum was then analyzed for kernel characteristics using the SKCS 4100 (Perten Instruments, Inc., Springfield, IL), based upon AACC Method 55-31. For each replication, measurements from 300 individual kernels were recorded. Sorghum kernels were analyzed for kernel weight by load cell, kernel diameter and moisture content by electrical current, and kernel hardness by pressure force.

SORGHUM MILLING

Commercial Flour

Both commercial varieties of sorghum flour, TV and AF, were obtained directly from each vendor (Twin Valley Mills, LLC, Ruskin, NE; Authentic Foods, Gardena, CA, respectively). To ensure a homogenous mixture, each flour was placed in a ribbon blender (Wenger Double Ribbon Stainless Steel Blender, Wenger Mfg., Sabetha, KS) and blended for 15 min. After mixing, the flour was randomly sub-divided into experimental units according to the statistical design. Each flour unit was stored in a re-sealable plastic bag until subjected to the pin milling treatments.

Laboratory Milling Methods

Cleaning

For all laboratory-milled flour treatments, white, food grade sorghum of Fontanelle variety D-1000-7 was used. The grain was cleaned on a dockage tester

(Carter-Day Company, Minneapolis, MN) using the previously described settings defined by FGIS for wheat cleaning.

Tempering

The sorghum samples milled to produce 60% and 80% extraction flours were tempered in order to facilitate the efficient separation of various sorghum kernel components. The grain was tempered to 16% moisture and held for 24 hrs prior to milling. The grain was added to the temper drum. As the drum rotated, the appropriate amount of water was slowly added. As the coefficient of friction increased, the product began to tumble and mix—evenly distributing the moisture among the kernels. The drum was sealed and allowed to tumble for 30 min, after which the tempered sorghum was transferred to sealed plastic bags and stored at room temperature.

60% Extraction Flour

Based upon preliminary studies comparing different milling methods, a Buhler Laboratory Mill (MLU-202, Uzwil, Switzerland) was chosen to initially mill the tempered sorghum samples. The general procedure followed is provided by AACC Method 26-22, Buhler Method for Hard Wheat (Figure 1) with the appropriate roll gap settings (Table 1).

A vibratory feeder (Syntron Power Pulse, FMC Technologies, Tupelo, MS) was calibrated to deliver 120 g of sorghum per minute to the mill. Prior to milling, the mill was warmed up by operating without grain for 30 min, followed by the milling of a 1000 g test sample. The mill was emptied and cleaned both prior to and after the milling of the test sorghum. After milling, bran—the coarse byproduct from the break portion of the mill— was set aside and considered to be feed stock. The shorts fraction—the coarse

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byproduct from the reduction portion of the mill—was sieved for 2 min on a Great Western Laboratory Sifter (Great Western Manufacturing, Inc., Leavenworth, KS) running at a 4" diameter throw at 180 rpm. The sieve frame used was clothed with a 150 µm aperture screen opening and removed any flour which tailed over the coarse reduction sieves. A total of 7 flour streams (including 3 each from the break and reduction systems, respectively, and the sifted shorts flour) were combined into the first flour extraction.

To generate additional flour, a series of additional grinding and separation procedures were conducted. Details regarding these steps have been omitted due to their proprietary nature.

Table 1. Roll Gap Settings for Buhler Mill				
	Break Rolls		Reduction Rolls	
	Left	Right	Left	Right
Inches	.00472	.00394	.00275	.00118
mm	.1	.08	.07	.03

CLEAN
WHEAT

1 BK	2 BK	3 BK	1 M	2 M	3 M
18 CORR.	22 CORR.	26 CORR.	SM	SM	SM
2:1	2:1	2:1	2:1	2:1	2:1

44 W	44 W	64 W	54 W	72 W	135 SS
135 SS	135 SS	135 SS	135 SS	135 SS	135 SS
			135 SS	135 SS	

FLOUR   FLOUR   FLOUR   FLOUR   FLOUR   FLOUR

BRAN   SHORTS
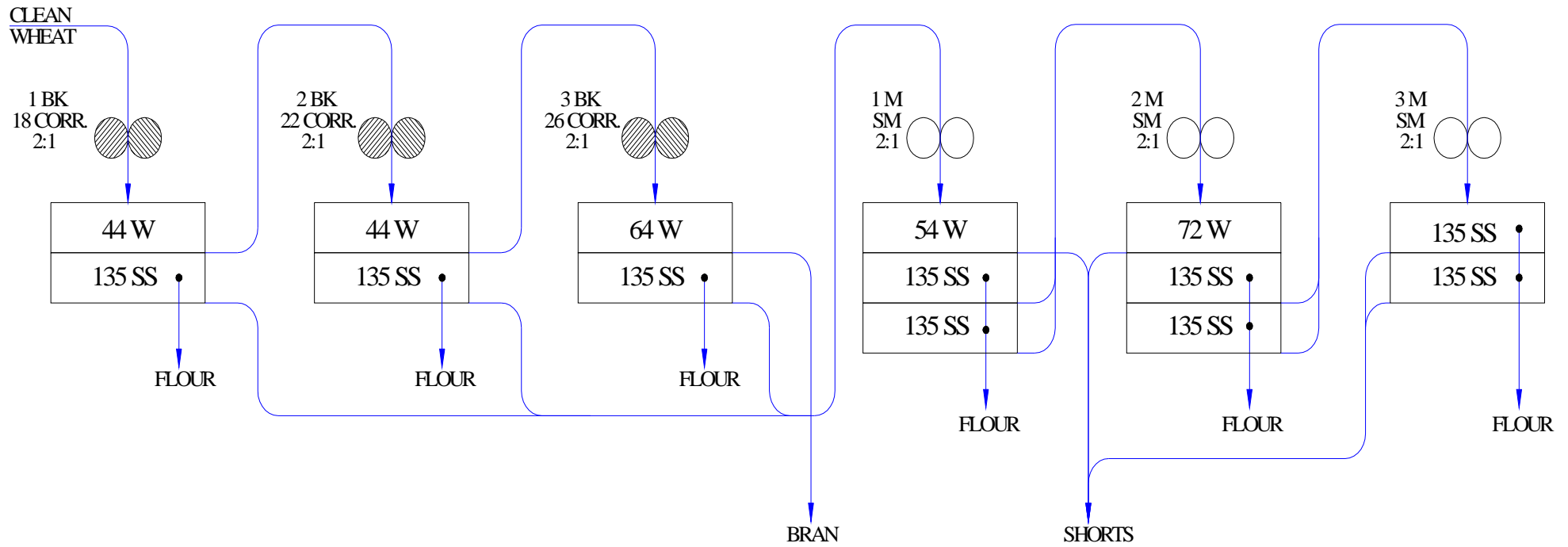
Figure 1. Milling flow for Buhler Mill used to mill 60% and 80% extraction sorghum flours.

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80 % Extraction Flour

To obtain a sorghum flour of 80% extraction, the same variety of sorghum was subjected to both the same initial Buhler milling and primary shorts sifting procedures. At this point, the three break, three reduction, and shorts flour streams were combined and set aside. To generate the additional flour necessary to reach 80% extraction, a series of additional grinding and separation procedures were conducted. Details regarding these steps have been omitted due to their proprietary nature.

Upon the final, the resulting flour was combined with all other flour streams in amounts necessary to achieve 80% extraction. The combined flour streams were blended for 30 min using the previously described flour-blending drum, randomly sub-divided into experimental units, and subjected to the appropriate pin milling treatments.

100 % Extraction Flour

Unlike the 60% and 80% extraction rate samples, there was no initial Buhler laboratory milling carried out in order to mill the 100% extraction flour. Additionally, the sorghum was not tempered prior to milling, as bran removal was not necessary. A series of grinding and separation procedures were conducted to produce the 100% extraction flour. Details regarding these steps have been omitted due to their proprietary nature. No by-products or co-products were manufactured from this milling process, thus generating a 100% whole, white sorghum product. The flour was blended for 30 min using the previously described flour-blending drum, subsequently sub-divided into experimental units, and later subjected to the appropriate pin milling treatments.

Pin Milling

In order to obtain flours of varying particle size with consistent composition, each experimental flour unit was subjected to one of three pin milling treatments that corresponded with the appropriate rotor speed: no pin milling ("No"), low speed pin milling ("Lo"), and high speed pin milling ("Hi"). Pin milling was carried out using an Alpine Pin Mill (160Z, Augsburg, Germany). The mill had both a static and dynamic set of rotor pins. The stationary pin set consists of four rows of 0.32 cm (1/8 in) diameter pins set in a circular pattern with the inside set having a 8.57 cm (3-3/8 in) diameter pattern and the outside set having a 14.6 cm (5-3/4 in) diameter pattern. The rotating pin set also had four rows of 0.32 cm (1/8 in) diameter pins with an inside diameter of 7.62 cm (3 in) and the outside set having a 15.88 cm (6-1/4 in) diameter pattern. The motor speed was 3440 rpm, with the belt drive and internal speed up drive resulting in a low rotor speed of 7,642 rpm and a high rotor speed of 14,620 rpm. (The inside/outside tip speed for the low rotor speed is 30.5/63.5 m/sec, and is 58.3/121.5 m/sec for the high rotor speed setting.) The resulting flours were then stored in re-sealable plastic bags, and stored for subsequent analyses.

SORGHUM FLOUR CHARACTERIZATION

Proximate Analyses

Moisture Content

The moisture contents of the flours were measured using the Association of Official Analytical Chemist (AOAC) approved method 930.15. The procedure determines the dry matter of the sample by oven drying at $135^\circ C$ for 2 hrs. Moisture is evaporated from the sample during the drying, and then dry matter is determined

gravimetrically as the residue remaining after drying. The moisture is then calculated by subtraction of dry matter from the whole sample.

Ash Content

The ash contents of the flours were measured using AOAC approved method 942.05. 2 grams of each sample was weighed into a porcelain crucible and placed in a temperature controlled furnace preheated to 600°C. The sample was held at this temperature for 2 hours. The crucible was then transferred directly to a desiccator, cooled, and then weighed. Ash content is reported as a percentage of the whole sample.

Protein Content

The protein contents of the flours were measured using AOAC approved method 990.03: Nitrogen Determination by Combustion. Nitrogen in the sample is freed by combustion at high temperatures in pure oxygen, and then measured by thermal conductivity. This value is converted to the equivalent protein by a numerical factor of 6.25.

Fat Content

The fat contents of the flours were measured using AOAC approved method 920.39. This method determines crude fat in the samples by ether extraction with subsequent solvent evaporation. The fat content is reported as a percentage of the original sample weight.

Fiber Content

The crude fiber contents of the flours were measured using the Ankom Method, based on AOAC 962.09. The Ankom Crude Fiber solvent solubilizes non-fiber

components of the flour, and then the sample is filtered, rinsed, and dried to determine the crude fiber content. Crude fiber is reported as a percentage of the original sample weight.

Physical Analyses

Total Starch Content

Total starch content was determined using the Megazyme Total Starch Assay Procedure (Amyloglucosidase/Alpha-Amylase), K-TSTA 05/2008, AACC Method 76.13 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). In this particular assay format, starch is first partially hydrolyzed and totally solubilized by cooking the flour in the presence of thermo-stable alpha-amylase. The resulting starch dextrins are then quantitatively hydrolyzed to D-glucose by the use of amyloglucosidase. Results were adjusted to dry basis values. The procedure was performed only on samples that had not been previously pin-milled.

Starch Damage

Starch damage was determined using the Megazyme Starch Damage Assay Procedure, K-SDAM 05/2008, AACC Method 76.31 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). In the procedure, damaged starch granules are hydrolyzed to maltosaccharides and alpha-limit dextrins through a highly controlled treatment with purified fungal alpha-amylase. This leads to nearly-complete solubilization of the damaged starch granules, while minimizing breakdown of undamaged granules. The reaction was terminated with dilute sulphuric acid. Aliquots were subsequently treated with excess levels of purified amyloglucosidase to give complete degradation of starch dextrins to glucose. The resulting solution is reacted with glucose oxidase/peroxidase

reagent, and the glucose concentration was determined colorimetrically. Reported values are presented as damaged starch as a percentage of flour weight on an "as is" basis.

Particle Size

A Beckman Coulter LS™ 13 320 Laser Diffraction Particle Size Analyzer (Beckman-Coulter, Inc., Miami, FL) was used to determine the particle size distribution of the flours. The dry powder system was used. The flour was placed into the load cell until it was approximately 2/3 full. The cell was then loaded into the Tornado™ Dry Powder Dispersing attachment for the instrument, and measurements were taken. The LS™ 13 320 uses light scattering properties to determine the particle size distribution.

Color

A HunterLab MiniScan (Model MS/S-4000S, Hunter Associates Laboratory Inc., Reston, VA) was used to measure the color of flour samples. The device was calibrated with a light trap and white tile provided by Hunter Associates Laboratory Inc. The type of illuminant used was C, average daylight, with a 10° Standard Observer. "L*", "a*", and "b*" values were given as output. "L*" is the measurement for lightness (0 = black and 100 = white). Red and green colors are indicated by the "a*" value (+a = red and –a = green). The "b*" value indicates yellow (+b) and blue (-b) colors.

Water Absorption Index

Water absorption characteristics of each flour were evaluated based upon AACC Method 56-11, Solvent Retention Capacity (SRC) Profile. SRC is defined as the weight of solvent held by flour after centrifugation, expressed as a percent of flour weight. Several solvents were used (deionized water, lactic acid, sucrose, sodium carbonate) in

order to establish wheat flour quality and functionality profiles. However, for this particular experimental objective, only deionized water was used. 50 mL centrifuge tubes were weighed prior to weighing 5.000 ± 0.050 g of flour into each tube. 25.00 mL of deionized water was added to each tube, the cap was secured, and the tube was vigorously hand-shaken for approximately 5 seconds to suspend the flour. The flour and water were allowed to solvate and swell for 20 min, with shaking occurring at 5, 10, 15, and 20 min for approximately 5 sec by use of a platform shaker (Innova 2000, New Brunswick Scientific Co., Inc., Edison, NJ). After shaking, the tubes were immediately transferred to a centrifuge (Eppendorf Centrifuge 5810, Hamburg, Germany) and centrifuged at 1000 x g for 15 min. After centrifuging, the supernatant was decanted at a 90° angle, and drained for 10 min over a paper towel. The tube was then weighed in order to determine the weight of the pellet. The weight of the gel was calculated by subtracting the weight of the tube after draining from the weight of the original empty tube. The % SRC was calculated as follows:

$$\% \text{ SRC} = \left[\frac{gel\ weight}{flour\ weight} \times \left(\frac{86}{100 - \% flour\ moisture} \right) - 1 \right] \times 100$$

SORGHUM BREAD PRODUCTION AND CHARACTERIZATION

Water Optimization

Prior to baking, the water addition necessary for each flour treatment was optimized by standardizing the batter consistency. For wheat bread, it is widely accepted that optimum water absorption may be determined with a Brabender farinograph or a mixograph. However, there are no such standard methods for water

absorption optimization for gluten-free breads. As a result, water optimization for this particular experiment was conducted by measuring the force necessary to extrude each batter using a texture analyzer, a method described by Schober and others (2005) and pioneered by Sanchez and others (2002). Preliminary results regarding the absorption characteristics of each flour were used to estimate the necessary water addition to achieve a standardized consistency. Thus, during testing, this pre-determined value, 5% more water, and 5% less water were used to interpolate the optimum amount of water for each of the flours. For the extrusion tests, the batters were prepared according to the formulation shown in Table 2, but yeast was omitted. The batter was mixed with a 300 W Kitchen Aid mixer (Ultra Power, St Joseph, MI) with a flat beater attachment for 30 sec at the lowest speed, and then scraped down from the sides of the bowl with a spatula. Mixing was continued for 90 more sec at level 2 out of 10. After resting for 5 min, the batter was loaded into the texture analyzer (TA-XT2, Stable Micro Systems, Godalming, United Kingdom), which was equipped with a 30 kg load cell, the forward extrusion cell, and a 10 mm nozzle. The extrusion force was measured at a test speed of 1.0 mm/sec over a distance of 20 mm. Pre-test and post-test speeds were 1.0 mm/sec and 10.0 mm/sec, respectively. The trigger force was 50 g. The averaged force after reaching a plateau was used an indicator of batter firmness. From this information, water addition was optimized for each flour, and is displayed in Table 3.

Table 2. Gluten-free sorghum bread formulation	
Ingredient	% Flour Basis
Sorghum Flour	70
Potato Starch	30
"Flour"	*100*
Salt	1.75
Sugar	1
HPMC	2
Active Dry Yeast	2
Water	Variable

Table 3. Water Addition (% FB) to bread formulation for each flour treatment			
Extraction Level	Pin Mill Treatment		
	No	Lo	Hi
60%	104	107	109
80%	113	116	118
100%	125	129	135
TV	106	107	108
AF	105	108	111

Breadmaking

The base formulation for the breads is shown in Table 2, as described by Schober and others (2007). Ingredients used were: sorghum flour, unmodified potato starch (Bob's Red Mill, Milwaukie, OR), iodized salt (Kroger, Cincinnati, OH), granulated sugar (Extra Fine, Great Value, Wal-mart Stores, Inc.,Bentonville, AR), hydroxypropyl methylcellulose (HPMC) (Methocel K4M, E 464, Dow Chemical Co., Midland, MI), and active dry yeast (Red Star Yeast, Milwaukee, WI). The sum of the sorghum flour and potato starch was interpreted as the flour weight basis. The addition of water to the formulation was modified for each flour in order to standardize the consistency of each batter, as previously described. Water amounts used are shown in Table 3.

The dried yeast was reactivated with 5 min of pre-hydration in the amount of water (30°C) appropriate for each flour treatment. The remaining dry ingredients were mixed separately, breaking up any clumps, and added to the yeast and water mixture. The batter was mixed with a 300 W Kitchen Aid mixer (Ultra Power, St Joseph, MI) with a flat beater attachment for 30 sec at the lowest speed, and then scraped. Mixing was continued for an additional 90 sec at level 2 out of 10. 250g of each batter was weighed into greased baking tins (9 cm x 15 cm x 5.5 cm) and proofed at 32°C and 85% relative humidity in a proofing cabinet (National Manufacturing Co., Lincoln, NE). Each batter

was proofed to height, corresponding to 1cm below the edge of the tin. Approximate proof time was about 40 min. After proofing, the batters were baked for 30 minin an electrically-powered reel-type test baking oven (National Manufacturing Co., Lincoln, NE) preheated to $232^{o}C$ ($450^{o}F$). Upon entering the oven, each batter was "steamed" by injecting 0.7L of water (by spraying with a spray bottle). After baking, the loaves were depanned and cooled for 1.5 hrs at ambient temperature.

Specific Volume

After cooling, loaves were weighed and loaf volume was measured by rapeseed displacement (AACC Method 10-05). Loaf specific volume (loaf volume [mL]/loaf weight [g]) was calculated.

Crumb Structure Evaluation

Once the specific volume of each bread was determined, the bread was sliced transversely using an in-house manufactured slice regulator and bread knife to obtain four slices of 25 mm thickness. The bread slices were assessed for crumb grain characteristics using a C-Cell Instrument (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). C-Cell uses high definition imaging and controlled illumination to obtain images, as illustrated by Figure 3. Chen and others (2007) showed that a C-Cell Instrument has the capability to determine important bread crumb attributes, including average cell diameter and volume, average cell wall thickness, average crumb fineness (number of cells/cm^2), and slice brightness.
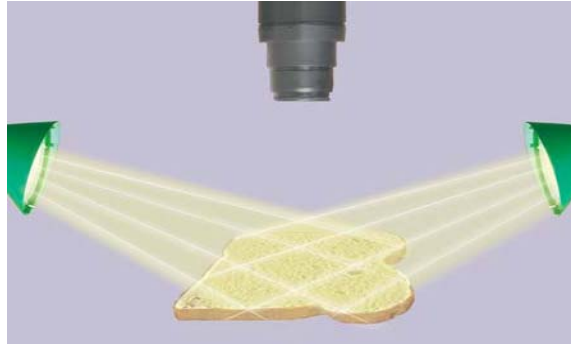
Figure 3. Illustration of C-Cell imaging process. From http://www.c-cell.info.

Texture Profile Analysis

Texture Profile Analysis (TPA) (Bourne 1978) of the crumb was performed on one slice from each loaf using a texture analyzer (TA-XT2, Stable Micro Systems, Godalming, United Kingdom) equipped with a 38 mm Perspex cylinder probe along with a 30 kg load cell. TPA was carried out with a constant speed of 2.0 mm/s (applying to the pre-test speed, test speed, and post-test speed) for a distance of 10.0 mm, corresponding to 40% compression of the 25 mm slices. There was a 5 second wait time between the first and second compression cycles; the trigger force was 20.0 g.

Statistical Design

Overall, this experiment utilized a split-plot design with a completely randomized main plot. A split-plot experiment has a main plot effect with larger experimental units, and is then subdivided into smaller experimental units to which a secondary treatment factor is applied. A split-plot design allows for experiments with a factor requiring a larger amount of experimental material to be paired with a second factor that requires a relatively smaller experimental unit. For this particular study, the main-plot effect was extraction level, with three replications under each main-plot level. Subsamples produced from each main-plot replication were randomly assigned to the sub-plot main effect, or pin milling treatment.

Proximate analyses and measurements of flour color were repeated in triplicates. Procedures for total starch, starch damage, particle size, and water absorption determination were performed in duplicates. Replications of each flour treatment were baked in duplicate loaves, and 8 slice views were evaluated for crumb characteristics.

All data were analyzed using SAS, Software Release 9.1.3 (SAS, Institute Inc., Cary, NC, 2003). When treatment effects were found significantly different, the least square means with Tukey-Kramer groupings were used to differentiate treatment means. A level of significance was observed at $\alpha \le 0.05$. Multiple linear regression was carried out to determine significance of interaction between variables. The level of significance is indicated in parentheses.

RESULTS AND DISCUSSION

SORGHUM KERNEL CHARACTERIZATION

Single Kernel Characterization System

Results for evaluation of sorghum kernel characteristics are shown in Table 4, including results for kernel hardness, weight, and diameter. All three characteristics have been used to determine grain quality (House 1985). The kernel hardness index of grain used in the present study (75.84) is aligned with results found by Fernholz and others (2008) for the same hybrid (75.0). In a study comparing gluten-free bread-making quality of different sorghum hybrids, Schober and others (2005) found a range of kernel hardness indices ranging from 72.1 to 82.7. Kernel hardness in sorghum has been linked to milling quality (Maxson and others 1971, Munck and others 1981, Rooney and Waniska 2000), as well as end-product quality, including cooked grain texture of sorghum (Cagampang and Kirleis 1984), porridge quality (Rooney and others 1986), and couscous quality (Aboubacar and Hamaker 1999).

Specifically related to gluten-free sorghum bread quality, Schober and others (2005) found the following correlations with SKCS kernel hardness: -0.682 (crumb hardness), 0.673 (crumb cohesiveness), 0.785 (protein), and 0.844 (starch damage). As a conclusion, Schober noted that kernel hardness was a "key element" for differences found in gluten-free bread quality when different sorghum hybrids were used. Unfortunately, kernel characteristics are unknown for the two varieties of sorghum used to mill the commercial sorghum flours. Such information could assist in better understanding milling and bread-making characteristics of these two flours.

Table 4. Kernel hardness, weight, and diameter using Single Kernel Characterization System for Fontanelle variety D-1000-7 sorghum grain			
	Single Kernel Characterization System Parameters		
	Hardness Index	Kernel Weight (mg)	Kernel Diameter (mm)
Fontanelle D-1000-7	75.84 ± 0.71	29.62 ± 0.28	2.37 ± 0.09

SORGHUM FLOUR CHARACTERIZATION

Chemical and Physical Analyses

Flour Composition

Significant differences were found ($p < 0.05$) in composition of sorghum flours evaluated (Table 5). Farrand (1972a) stated that controlled measures taken to alter levels of starch damage are unlikely to significantly affect any flour constituents other than the physical state of the starch. As such, results for compositions of all flours prior to pin milling are shown. Specific results include protein, fat, ash, moisture, fiber, and total starch. Among laboratory-milled flour samples, protein content significantly increased with increasing extraction level. Among commercially-milled samples, TV had the lowest protein content, and the protein content of AF fell between 80% and 100% extraction flours. For fat and ash contents, TV had the highest levels of both component, followed by 100% extraction and AF. 60% and 80% extraction flours had the lowest levels of fat and ash among all flours studied. Measurements of fiber content showed significant differences in each sample. 100% extraction flour had the highest fiber content, followed by both commercial flours; 60% extraction flour had the lowest fiber content. Increases in each of these four components are associated with increases in extraction rate. Higher extraction flours contain higher concentration of the aleurone layer and the peripheral endosperm in the flour, which are the components of the caryopsis that are the main sources of fat, ash, protein, and fiber (Rooney and Clark 1968). Similar results were found in a study on the effect of extraction level of wheat flour on tortilla texture (Ramirez-Wong and others 2007). Effects of these flour components on bread-making are discussed further where applicable.

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Significant differences for moisture content were found (p<0.05) among various flour extractions and types (Table 5). Values ranged from 8.49% (AF) to 12.22% (60% extraction). Direct comparisons among laboratory-milled flours to commercially-milled samples cannot be made, as processing, milling, and storage conditions of the commercial samples are unknown. However, differences among laboratory-milled samples may be due to milling processes necessary to achieve the specific and desired extraction rate. Farrand (1972a) noted a significant, inverse correlation between moisture and starch damage. The mechanical force necessary to produce flours of greater starch damage resulted in heat generation on the milling rolls, and thus caused a significant moisture loss in the flour.

Flour moisture is typically an indication of flour quality and has an impact on functionality in specific products. For example, it is recommended that flour used to produce cookies and crackers be at a moisture content of 13% (± 0.5%) in order to obtain proper texture (Haynes and others 2009). The role of flour moisture in product quality is demonstrated by changes in water absorption (Regnier and others 2004). Chung and others (1978) found that rheological dough properties (specifically mixograph absorption) were affected by moisture content of flour samples. The same study showed that flour moisture content had a significant effect on loaf volume.

Significant differences were found (p<0.05) for total starch content between flour treatments (Table 5). Among laboratory-milled sorghum flour samples, total starch content ranged from 71.16% (100% extraction) to 85.71% (60% extraction), with total starch content decreasing with an increasing extraction rate. Both commercial flour samples were found to be not significantly different from the 80% extraction flour

sample. The decrease in total starch content noted with increasing extraction rate can be attributed to dilution from other caryopsis components, including the pericarp, aleurone layer, germ, as well as the endosperm which also contains protein (Wall and Blessin 1969).

Table 5. Compositions of laboratory- and commercially-milled sorghum flours						
Flour Extraction	Protein	Fat	Ash	Fiber	Moisture	Total Starch
60	7.74 ± 0.09^d	1.97 ± 0.19^c	0.90 ± 0.11^c	0.78 ± 0.07^e	12.22 ± 0.62^a	85.71 ± 1.61^a
80	9.19 ± 0.25^b	2.22 ± 0.13^c	1.01 ± 0.05^c	1.00 ± 0.07^d	11.32 ± 0.59^{ab}	81.54 ± 2.22^{bc}
100	9.81 ± 0.33^a	3.03 ± 0.09^b	1.44 ± 0.03^b	2.14 ± 0.02^a	9.11 ± 1.23^c	71.16 ± 1.79^d
TV	6.91 ± 0.01^e	3.80 ± 0.13^a	1.65 ± 0.01^a	1.32 ± 0.02^c	10.24 ± 0.03^{bc}	82.98 ± 1.69^b
AF	8.47 ± 0.01^c	3.04 ± 0.03^b	1.34 ± 0.01^b	1.50 ± 0.03^b	8.49 ± 0.02^c	79.70 ± 2.05^c
For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05).						

Flour Particle Size

Significant differences in flour particle size were found (p<0.05) among all flours studied, both within extraction level and pin-milling treatment (Table 6). Flour particle size for each flour treatment at the 90 volume percent (d90) was reported, which indicated that 90 percent of the flour particles were less than the stated size. Additionally, measurements of mean flour particle size for each flour treatment are given. For both variables, data for the particle size of commercial sorghum flour TV is not shown, as the sample was unable to be determined due to equipment malfunction/sample incompatibility. The sample had issues with clumping, despite several attempts to correct the issue. Particle size (d90) ranged from 120.10 μm (AF, high-speed pin-milling) to 256.96 μm (100% extraction, no pin-milling). Mean particle size ranged from 54.99 μm (60% extraction, high-speed pin-milling) to 129.14 μm (100% extraction, no pin-milling). Among the laboratory-milled sorghum flour samples, particle size was significantly different among flour samples within each pin-milling treatment (p<0.05); particle size decreased with increasing pin mill speed (Figure 4). Within the no pin-milling and low-speed pin-milling treatments, particle size significantly increased with increasing extraction rate. However, within the high-speed pin-milling treatment, flour particle size was not considered to be significantly different among varying extraction rates. The same trends in results and significant differences were found in the data for mean particle size.

Flour particle size is an indication of the degree of fineness of a flour sample, as well as its total exposed surface area (Pratt 1978). It appears that the present pin-milling treatments were successful in reducing flour particle size of the flours studied. However,

of particular interest are the particle size results for AF. There was not a significant decrease in particle size found between the no pin-milling and low-speed pin-milling treatments, and while the high-speed pin-milled AF flour was found to have a significantly lower particle size than the no or low-speed pin-milled flours, the decrease was to a lesser degree than seen for the laboratory-milled flours. Oh and others (1985) found that pin-milling reduced particle size for both hard and soft wheat flours. However, the particle size decreased only slightly for the soft wheats, but much more so for the hard wheats studied. Even though there is little known about the sorghum grain used to mill the AF flour, these results may indicate that AF is a soft sorghum flour. Farrand (1972a) found that hard wheat produced larger flour particles than soft wheat. Hard and soft wheats, and perhaps sorghums, fracture differently during the milling process, causing variation in particle size (Simmonds 1974). Found to be a varietal trait, grain endosperms that fracture more readily (softer endosperms) produce finer flour than those with harder endosperms. Additionally, these varietal differences carry over to pin-milling and additional methods of particle size reduction (Yamazaki and Donelson 1972).

As exhibited by this study, flour particle size can be reduced by regrinding a sample. However, an additional reduction of particle size is typically associated with an increase in starch damage (Gaines and others 1988). As discussed in more detail later, it was shown that flour particle size exhibits independent effects on baking and bread quality (Pratt 1978). However, because a decrease in flour particle size is often accompanied by an increase in starch damage, it has been suggested that damaged

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starch may have greater effects on baked product quality than particle size alone (Shelton and D'Appolonia 1985, Kurimoto and Shelton 1988).

	Particle Size Distribution (d90)			Mean Particle Size			Starch Damage		
	Pin Milling Treatment			Pin Milling Treatment			Pin Milling Treatment		
	No	Lo	Hi	No	Lo	Hi	No	Lo	Hi
60%	168.84 ± 2.16^{Ac}	140.26 ± 0.69^{Bc}	114.60 ± 2.78^{Ca}	98.76 ± 0.78^{Ac}	75.57 ± 1.96^{Bc}	54.99 ± 1.80^{Cb}	9.90 ± 0.14^{Cc}	11.10 ± 0.26^{Bc}	13.06 ± 0.41^{Ac}
80%	211.51 ± 14.57^{Ab}	163.05 ± 9.00^{Bb}	119.53 ± 2.50^{Ca}	117.97 ± 3.11^{Ab}	88.36 ± 4.65^{Bb}	57.96 ± 0.99^{Cab}	13.92 ± 0.16^{Ca}	16.07 ± 0.38^{Ba}	18.07 ± 0.15^{Aa}
100%	256.96 ± 22.55^{Aa}	197.29 ± 22.55^{Ba}	117.93 ± 7.17^{Ca}	129.14 ± 9.67^{Aa}	100.34 ± 9.32^{Ba}	55.21 ± 3.31^{Cb}	12.18 ± 0.61^{Bb}	11.86 ± 0.58^{Bb}	16.51 ± 3.43^{Ab}
TV							9.60 ± 0.18^{Cd}	11.16 ± 0.03^{Bc}	12.76 ± 0.22^{Ac}
AF	132.07 ± 1.22^{Ad}	132.18 ± 3.36^{Ac}	120.10 ± 6.20^{Ba}	67.61 ± 0.95^{Ad}	68.28 ± 2.89^{Ac}	71.68 ± 19.55^{Aa}	8.35 ± 0.08^{Ce}	8.96 ± 0.12^{Bd}	9.73 ± 0.07^{Ad}

Table 6. d90 particle size distributions, mean particle size distributions, and starch damage of sorghum flours milled to varying extractions and pin-milling treatments

For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different within each variable (p>0.05).
For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different within each variable (p>0.05).

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Starch Damage

For all flours studied, significant differences in starch damage were found (p<0.05) within each extraction level (Table 6); starch damage increased with increasing speed of pin-milling. Among laboratory-milled samples, values ranged from 9.90% (60% extraction, no pin-milling) to 19.12% (100% extraction, high-speed pin-milling). Within each pin-milling treatment of the laboratory-milled samples, flours were found to be significantly different from one another. However, there was an inconsistent and unexpected relationship between extraction level and starch damage. For the no- and low-speed pin-milling treatments, the 80% extraction flours had the highest starch damage levels (13.92%, 16.07% respectively). While it was thought that higher starch damage would result from a greater degree of regrinding (due to increasing the extraction rate, i.e. 100% extraction rate), the observed opposite effect can best be explained by a variation in milling method. As described previously, the 100% extraction flours were milled on Hal Ross roller mills, and the lower extraction flours were milled on Buhler Laboratory milling equipment. While every effort was made to standardize the milling procedures for each treatment, roll pressure, roll surface, and roll speed differential have been found to be responsible for producing damaged starch (Jones 1940). Overall, the commercially milled samples exhibited the lowest levels of starch damage. However, the milling methods used to prepare these samples are unknown. As such, direct comparisons between all treatments are ineffective.

As grain endosperm is gradually reduced into flour during milling, intact starch granules become damaged, leaving starch granules that have been fractured, shattered, or chopped (Chen and D'Appolonia 1986). It appears that grain hardness is a

major contributor to starch damage that results from milling. Hard wheats produce larger flour particles with a higher degree of starch damage when compared to soft wheats (Farrand 1972a). These differences are due, in part, to the fracture characteristics of the grain during milling (Simmonds 1974). In soft wheat, the starch granules are loosely bound within the kernel and are easily released. However, the starch granules of hard wheat are tightly bound within the protein matrix, and are much more susceptible to damage as the endosperm is fractured (Evers and Stevens 1985).

Reported as early as 1925, damage to the structural integrity of a starch granule as a result of grinding alters flour characteristics (Alsberg and Griffing 1925). Injured starch granules demonstrate an increase in water absorption and conversion of starch by enzymes (Kurimoto and Shelton 1988). During breadmaking, damaged starch will compete for water with other flour components, most notably protein. Therefore, bread properties will be affected if starch damage is excessive (Pyler 1988). Farrand (1972b) found that an increase in starch damage above "acceptable levels" for any given protein content caused a corresponding increase in water absorption, as well as a decrease in loaf volume. Specific effects of starch damage on bread produced in the present study will be discussed in detail.

Color

L* Values

Results for L* values for all flour samples studied are shown in Table 7, with the exception of TV, as an equipment failure prevented evaluation of this particular sample. Values ranged from 82.56 (100% extraction, no pin-milling) to 89.46 (60% extraction, high-speed pin-milling). The L* value indicates the measure of lightness of a sample

and is considered to be an expression the sample's whiteness. The value ranges from 0 (black) to 100 (perfect white), with higher values indicating brighter samples (Hutchings 1994, Kurimoto and Shelton 1988). Within flour samples subjected to no additional pin-milling, L* values decreased significantly (p<0.05) with increasing extraction rate. For flours milled to 60% and 80% extraction, the low- and high-speed pin-milled samples are not considered to be significantly different from one another in degree of whiteness. However, 100% extraction flours within the same pin-milling treatments were considered to be significantly lower in L* values when compared to other extraction rates. 100% extraction flour and AF were not considered to be significantly different among all extraction rates in relation to L* values.

Previous studies have shown that extraction rate, particularly affected by fiber content, has an effect on the brightness of a sample. Oh and others (1985) noted a decline in flour brightness when wheat flour extraction was increased by 8%. Fiber and particle size significantly contributed to L* (and overall flour color) in this particular study (p<0.0001). Particle size has been shown to have an impact on flour color, and particularly L* values. Kurimoto and Shelton (1988) examined the effect of wheat flour particle size on flour attributes. Results for L* values showed a significant increase with decreasing particle size, with a correlation coefficient of -0.98 (p<0.01), suggesting that finer flour appears to be brighter or whiter.

a* Values

Results for a* values for all flours studied are shown in Table 7, with the exception of TV for the aforementioned reason. Values ranged from -0.22 (60% extraction, high-speed pin-milling) to 0.68 (100% extraction, high-speed pin-milling). The

a* value is a measure of the degree of redness or greenness of a sample, ranging from -100 to +100 (Hutchings 1994). A positive value indicates redness, and a negative value expresses greenness. A value of 0 is indicative of a grey sample (Kurimoto and Shelton 1988). 60% and 80% extraction flours had significantly lower a* values than 100% extraction and AF in each pin-milling category ($p<0.05$). For flours milled to 80% and 100% extraction rates, degree of pin-milling had no significant effect on a* values. However, for the 60% extraction flour treatments, flours pin-milled at low and high-speeds had significantly lower a* values than flour that was not pin-milled. Similar results are seen for AF, but flours pin-milled at low and high-speeds had significantly higher a* values than flour that was not pin-milled. While results for the collection of samples exhibited both positive and negative values, overall, the values were close to zero, and indicate a grey appearance.

Extraction rate also seems to have an impact on a* values of flour samples. Ash content—an indication of bran contamination in flour—has been correlated with flour color (Kim and Flores 1999). A correlation coefficient of 0.75 was observed in this study between fiber and a* values. Ramirez-Wong and others (2007) found significant differences in a* values with variation in extraction rate. Specifically, as the rate of extraction increased, the a* values decreased (became more negative). However, in the aforementioned study by Kurimoto and Shelton (1988), samples of varying extraction rates and particle sizes showed no significant change with respect to a* values. It is important to note that of the flours examined in the 1988 study, the ash contents were not significantly different. However, the present study showed significant increases in ash content with increasing extraction rate.

b* Values

Results for b* for all flours studied are shown in Table 7, with the exception of TV, for the aforementioned reason. Values ranged from 11.56 (60% extraction, high-speed pin-milling) to 15.17 (AF, no pin-milling). The b* value is a measure of the degree of yellowness (positive values) or blueness (negative values) of a sample, ranging from -100 to +100 (Hutchings 1994). A value of 0 is indicative of a grey sample (Kurimoto and Shelton 1988). For each extraction rate, b* values were not significantly affected by degree of pin-milling ($p<0.05$). AF had the highest b* values among all flour treatments, followed by 100% extraction flours. 60% and 80% extraction flours were not significantly different with the exception of flour subjected to high-speed pin-milling; high-speed pin-milled 80% extraction flour had a significantly higher b* value than 60% extraction flour of the same pin-milling treatment.

Little research was found on the effect of flour particle size on color of a sample, specifically on yellowness and b* values. However, Kurimoto and Shelton noted a significant decrease as the sample particle size decreased, with a correlation coefficient of 0.99 ($p<0.01$). However, this same finding was not repeated in the present study. It appears that extraction rate has the most significant effect on the relative yellowness or blueness of the sample.

The L*, a*, and b* values together determine flour color, the dominant factor in determining crumb color. In fact, Pomeranz (1960) observed that flour color was correlated with crumb color with a coefficient of 0.987. As discussed within each attribute of color, flour is influenced by composition, and most notably freedom from

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bran particles (Pyler 1988). Color, either in crumb or crust, is a central characteristic for

acceptance of baked products (Sabanis 2009).

	L			a			b		
	Pin Milling Treatment			**Pin Milling Treatment**			**Pin Milling Treatment**		
	No	**Lo**	**Hi**	**No**	**Lo**	**Hi**	**No**	**Lo**	**Hi**
60%	87.79 ± 1.86^{Ba}	88.19 ± 1.50^{ABa}	89.46 ± 1.29^{Aa}	0.04 ± 0.22^{Ab}	-0.18 ± 0.15^{Bb}	-0.22 ± 0.11^{Bb}	11.82 ± 0.44^{Ac}	12.46 ± 1.00^{Abc}	11.56 ± 1.05^{Ac}
80%	86.01 ± 2.09^{Bb}	87.49 ± 1.26^{ABa}	88.66 ± 0.95^{Aa}	-0.05 ± 0.09^{Ab}	-0.03 ± 0.15^{Ab}	-0.16 ± 0.07^{Ab}	12.62 ± 1.02^{Ac}	12.18 ± 0.47^{Ac}	12.51 ± 1.40^{Ab}
100%	82.56 ± 0.51^{Bc}	83.58 ± 1.12^{Bb}	85.45 ± 0.69^{Ab}	0.56 ± 0.09^{Aa}	0.42 ± 0.13^{Aa}	0.52 ± 0.18^{Aa}	13.55 ± 0.42^{Ab}	13.26 ± 0.64^{Ab}	13.07 ± 0.64^{Ab}
TV									
AF	83.35 ± 2.25^{Bc}	83.80 ± 0.86^{ABb}	85.17 ± 0.73^{Ab}	0.39 ± 0.14^{Ba}	0.64 ± 0.26^{Aa}	0.68 ± 0.12^{Aa}	15.17 ± 0.83^{Aa}	14.26 ± 0.69^{Aa}	14.66 ± 0.73^{Aa}

Table 7. L, a, and b values of sorghum flours milled to varying extractions and pin-milling treatments

For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different within each variable (p>0.05).
For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different within each variable (p>0.05).

Water Absorption

Significant differences were found ($p < 0.05$) for water absorption values among flours evaluated (Table 8). Among the laboratory-milled samples, values for water absorption (expressed as a percentage of original flour weight) ranged from 117.12% (60% extraction, no pin-milling) to 146.78% (80% extraction, high-speed pin-milling). Within each pin-milling treatment, 60% extraction samples had the lowest values for water absorption; 80% and 100% extraction samples were not considered to be statistically different from one another ($p < 0.05$). With the exception of the commercial flour samples, water absorption increased significantly with increasing pin-milling speed ($p < 0.05$). For TV, only a slight increase in water absorption was seen for the high-speed pin-milled sample. For AF, samples did not significantly increase in water absorption with increasing pin mill speed ($p < 0.05$). This can perhaps be attributed to the earlier-speculated soft nature of the grain used to produce AF, causing the flour to have non-significant decreases in particle size and only slight increases in starch damage with increasing pin-milling speed.

Effect of Particle Size on Water Absorption

Torres and others (1994) tested decorticated sorghum flours of varying particle sizes in wheat flour tortillas at several replacement levels. It was found that particle size distribution of the sorghum flour was an important factor affecting water absorption of the doughs. More specifically, pin-milled sorghum flour, as opposed to hammer-milled sorghum flour, had a higher absorption than the flour used in the control dough. Oh and others (1985) found that flours with small particle size required more water during the production of noodles. This finding is likely due to the increased surface area of finer

particle size flours. In this particular study, no statistically significant correlation was observed between particle size and water absorption for simple linear regression. However, analysis of multiple linear regression showed that flour particle size did significantly contribute to water absorption (p<0.0001). As discussed earlier, a decrease in particle size leads to an increase in starch damage, indicating that the two variables are interrelated. The lack of a significant one way interaction between particle size and water absorption may suggest that several flour characteristics are responsible for sorghum flour water absorption.

Effect of Starch Damage on Water Absorption

A correlation coefficient of 0.87 (p<0.0001) was found between water absorption and starch damage. This was higher than the correlation coefficient of 0.76 that was found between water absorption and starch damage of wheat flour by Kurimoto and Shelton (1988), indicating that perhaps there is a stronger relationship between the two variables in sorghum flour. Oh and others (1985) observed an increase in water absorption in wheat flour samples with higher amounts of starch damage. Farrand (1972a), who studied the influence of starch damage and particle size on the characteristics of wheat flours for breadmaking, noted that an increase in starch damage resulted in an increase in absorption. Hatcher and others (2009) concluded that as starch granules become damaged through processing, the surface area increases, which corresponds to an increase in the water hydration capacity of the granules. This causes competition between starch granules and proteins within the flour for available water, and therefore has an impact on the viscoelastic behavior of the dough/batter.

Effect of Fiber on Water Absorption

Fiber was a significant predictor of water absorption ($p < 0.0001$). The increase in water absorption in flour due to the addition of dietary fiber has been widely reported. Pomeranz and others (1977) and Gan and others (1992) found that bran particles from brown rice flour and buckwheat flour, respectively, swelled extensively due to high concentrations of fiber in the products. Similar results were noted for wheat flour, with Gomez and others (2003) noting that water absorption increased with the addition of dietary fiber. Pomeranz and others (1977) observed an increase in water absorption that coincided with an increase in extraction rate, most likely due to higher protein and fiber contents found in higher extraction flours. In a study on the effects of wheat fiber addition to gluten-free bread, Sabanis and others (2009) found that additional water was required to produce an easy-to-handle dough at wheat fiber addition levels of 3, 6, and 9 g/100 g.

As fiber is a highly water-binding macromolecule, it competes with starch and other flour components for water (Collar and others 2006). The extent of these alterations in water absorption seems to depend on the specific structure of the fiber, and was likely due to the number of hydroxyl groups present in fiber molecules. These hydroxyl groups are responsible for water interaction through hydrogen bonding (Lazaridou and others 2007, Sabanis and others 2009). Sorghum is rich in the insoluble fiber component, glucurono arabinoxylan, while barley is rich in soluble beta-glucans and wheat fiber is made up of soluble and insoluble arabinoxylans (Taylor and Dewar 2001). The differences in molecular weight and branching structure between these fiber components may shed insight into the effect of sorghum fiber on sorghum flour water absorption compared to other cereals.

Table 8. Comparison of water absorption for sorghum flours milled to varying extractions and pin-milling treatments			
Water Absorption			
Flour Extraction	Pin-milling Treatment		
	No	Lo	Hi
60%	117.12 ± 2.64^{Bb}	115.84 ± 4.10^{Bb}	125.76 ± 2.31^{Ab}
80%	135.81 ± 2.15^{Ca}	142.52 ± 2.62^{Ba}	146.78 ± 1.58^{Aa}
100%	132.97 ± 2.85^{Ca}	140.16 ± 2.05^{Ba}	144.66 ± 1.64^{Aa}
TV	100.06 ± 1.62^{Bc}	99.44 ± 0.83^{Bc}	104.51 ± 1.45^{Ac}
AF	97.62 ± 1.01^{Ac}	98.07 ± 0.82^{Ac}	100.11 ± 2.02^{Ad}

For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05).
For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05).

BREAD CHARACTERIZATION

Specific Volume

Significant differences were noted (p<0.05) for the specific volume of breads produced with all sorghum flours studied (Table 9). Values ranged from 2.01 mL/g (100% extraction, no pin-milling) to 2.54 mL/g (60% extraction, low-speed pin-milling). Overall, specific volume was significantly affected by both extraction level and pin-milling treatment. Within all pin-milling treatments, breads produced from 60% extraction flour had significantly higher specific volumes when compared to all other extractions and flour types. Additionally, breads produced from 60% extraction flour subjected to low-speed pin milling showed the highest specific volumes, followed by high-speed and no pin-milling, respectively. Specific volumes of breads produced from 100% extraction flour subjected to low- and high-speed pin milling were significantly higher than non pin-milled flour of the same extraction. Breads produced from TV subjected to high-speed pin-milling showed specific volumes significantly higher than low-speed or non-pin milled flour, respectively. Breads produced from 80% extraction flour and AF were not significantly affected by pin-milling.

Effect of Fiber on Specific Volume

Specific volume has been shown to be affected by many factors, including dough composition (including amounts of water, fiber, starch, protein, processing aids, etc.), processing conditions, and dough rheology—all properties that impact gas retention capabilities. While there is a nutritional benefit to the incorporation of dietary fiber into gluten-free products (as well as other baked goods), this is met with the limitation of decreased volume (Krishnan and others 1987; Chen and others 1988; Pomeranz and

others 1977; Sievert and others 1990). In the present study, fiber contributed significantly to specific volume ($p<0.0001$). In general, flours with lower extraction rates produced breads with significantly higher loaf volumes. Research by Gan and others (1989, 1992) stated that the non-endosperm components of wheat (germ, bran, and epicarp hairs) were responsible for the depression of loaf volume. Additionally, it was observed that due to concentrated fiber content, bran particles from brown rice flour and buckwheat flour swelled extensively during dough mixing, causing a weakened structure and a decreased volume of bread (Pomeranz and others 1977). Gan and others (2005) suggested that bran particles would disturb the homogeneity of the starch gel and prevent uniform gas cell formation. In wheat bread, the dough and bread structures are stabilized and strengthened by a gluten network, yet decreased volume is still seen when fiber is incorporated. It was therefore hypothesized by Moore and others (2004) that this deleterious effect on volume could be expected to be even worse in gluten-free baked products. These results add validity to the observations in the present study that show a decrease in specific volume with an increase in extraction rate (i.e. an increase in fiber content).

Additionally, particle size of the fiber has been shown to affect specific volume. Moder and others (1984) demonstrated that finely ground bran produced breads with higher loaf volumes than did coarse bran. However, Lorenz (1976) studied the effect of bran particle size on loaf volume; it was found that fine triticale bran produced bread loaves with similar loaf volumes, but better grain characteristics (although specific grain characteristics were not defined). These previous studies may assist in explaining the significant increase in specific volume with an increase in degree of pin-milling seen in

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breads produced with 100% extraction flour and TV—two samples on the higher end of fiber content.

Effect of Starch Content on Specific Volume

While increased fiber content is certainly a contributing factor of observed decreases in specific volume, a decrease in starch content (as a result of increased extraction rate) must also be considered responsible for alterations in loaf quality. The present study showed that starch content significantly contributed to loaf volume (p<0.0015). Moore and others (2004) found that starch-based gluten-free breads achieved higher volumes. In two studies from Olatunji and others (1991, 1992), favorable results were achieved for gluten-free bread by using a 70:20:10 blend of decorticated sorghum flour, gelatinized cassava starch, and raw cassava starch, respectively. Additionally, the use of various starches (modified sorghum, waxy sorghum, corn, cassava, arrowroot, potato) in combination with methylcellulose improved oven rise and crumb structure of gluten-free bread (Taylor and others 2006).

Starch has several hypothesized roles in improving gluten-free sorghum bread. Some researchers have suggested a dilution effect. Endosperm and bran particles in sorghum flour become diluted by any added starch, disturbing the homogeneity of the starch gel and impeding gas cell formation (Gan and others 2005). Schober and others (2005) used similar reasoning to hypothesize that gluten-free breads produced from whole-grain flours (i.e. lower starch and higher fiber contents) would have lower loaf volume than pure starch breads.

Effect of Particle Size on Specific Volume

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Flour particle size has also been shown to affect overall baked product quality, but specifically loaf volume. For this study, particle size was shown to be a significant predictor for specific volume ($p<0.0001$). Yamazaki and Donelson (1972) reported a correlation coefficient of -0.94 between median diameter of patent flour and cake volume. A similar relationship was noted by Chaudhary and others (1981) with a correlation coefficient of -0.85 for the same relationship. Additionally, Miller and others (1967) found that cake volume improved as flour particle size decreased as a result of pin milling to alter the flour properties. Such results may provide some rationale as to why significant increases in specific volume were noted for breads produced from 60% extraction, 80% extraction, and TV that had been subjected to some degree of pin-milling to reduce flour particle size.

Flour particle size does appear to have an impact on loaf volume, the variable was not significantly correlated with specific volume in the present study ($p>0.05$). Several researchers have noted that flour particle size on its own is not the cause for alterations in specific volume. In the same previously mentioned study by Chaudhary and others (1981), flour was milled and refined using air classification and wet-fractionation to minimize differences in particle size; cake volumes still differed significantly. As such, flour particle size was not considered to be the primary driver of volume. Furthermore, it was suggested that the characteristic governing flour granulation may be responsible. Research from Yamazaki and Donelson (1972) supports this hypothesis by suggesting that flour particle size is not a sole contributor to particle size. In their study, wheats of varying inherent granularity were milled in a uniform manner; grains that granulated more easily and finely produced flours that

showed greater cake volume potential. These previous findings help to discern why more drastic differences in specific volume were not observed for breads produced from flours of differing particle size.

Effect of Starch Damage on Specific Volume

Because increased starch damage is a result decreasing flour particle size, its synergistic effects with particle size must not be ignored; Farrand (1972b) observed that loaf volume and crumb structure were significantly correlated with variations in starch damage. In the study at hand, starch damage was a significant predictor for loaf volume ($p<0.0001$). As mentioned earlier, specific volumes of the breads studied did not increase to the degree expected with increases in starch damage. In fact, for breads produced from 60% extraction flour, specific volume actually decreased between low- and high-speed pin milling. Miller and others (1967) reported that excessive pin-milling caused a considerable increase in starch damage which negatively affected cake quality. Excessive starch damage leaves swollen starch granules susceptible to attack by alpha-amylase (Tipples 1969). An increase in the hydrolysis of starch by alpha-amylase will decrease the viscosity of the dough/batter matrix and affect end-product quality. The result is a sticky, heavy crumb texture with low volume (Evers and Stevens 1985). Since it appears that starch damage may have a diminishing return effect on specific volume, further investigation is needed to determine the appropriate level for production of gluten-free sorghum bread.

Table 9. Comparison of specific volumes of bread produced from sorghum flours of varying extractions and pin-milling treatments			
Specific Volume (mL/g)			
Flour Extraction	**Pin-milling Treatment**		
	No	**Lo**	**Hi**
60%	2.40 ± 0.07^{Ba}	2.54 ± 0.09^{Aa}	2.45 ± 0.05^{ABa}
80%	2.22 ± 0.10^{Ab}	2.27 ± 0.09^{Ab}	2.20 ± 0.19^{Ab}
100%	2.10 ± 0.12^{Bbc}	2.21 ± 0.16^{Ab}	2.22 ± 0.08^{Ab}
TV	2.06 ± 0.07^{Bc}	2.15 ± 0.06^{ABb}	2.23 ± 0.11^{Ab}
AF	2.01 ± 0.07^{Ac}	2.03 ± 0.08^{Ac}	2.11 ± 0.08^{Ab}
For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05). For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05).			

CRUMB STRUCTURE EVALUATION

Cell Diameter & Volume

Results for cell diameter in breads produced with all sorghum flours studied are shown in Table 10. Values for cell diameter ranged from 2.07 mm (AF, low-speed pin-milling) to 2.68 mm (100% extraction, low-speed pin-milling). Values for cell volume ranged from 7.68 mm^3 (AF, no pin-milling) to 11.30 mm^3 (100% extraction, low-speed pin-milling). Within each flour extraction level and flour type, degree of pin milling did not significantly affect bread cell diameter or volume. Breads produced from 100% extraction flour tended to have significantly larger cell diameters and volumes than those made with lower extraction flours. Breads produced from AF had the lowest cell volumes among flours subjected to low-speed pin-milling. Breads produced from 60% extraction rate flour and commercial flours AF and TV tended to have significantly lower cell diameters than other samples within the same pin-milling treatments.

In wheat bread, the extent to which cells are formed is a function of the protein-starch interactions (specifically from gluten) that provide viscoelastic properties to the dough (Gan and others 1989). As gluten-free bread lacks the means necessary to produce such a network, another mechanism is utilized to form gas cells. Air cells, or alveoli, are created during mixing. Carbon dioxide, which is produced as a byproduct of yeast fermentation, diffuses into these air cells, causing them to expand (Gan and others 1995). Overall, a smaller cell diameter is indicative of a smaller cell volume. In fact, in the present study, the correlation coefficient between cell diameter and cell volume was 0.97 (Table 13). Quality white pan breads are characterized by small, elongated gas cells with thin cell walls (Hayman and others 1998). Smaller cells,

whether defined by volume or diameter, are desirable in gluten-free bread products, as greater numbers of small gas cells have been found to produce loaves of higher specific volumes (Gallagher and others 2003). Larger cell diameters are typically indicative of gas cell coalescence. Ahlborn and others (2005) found that gas cell coalescence diminishes the presence of a web-like structure which, if achievable in gluten-free bread, improves both visual and eating properties of the product. In this particular study, starch damage and flour particle size were both significant predictors of cell diameter and volume ($p < 0.0001$). As discussed previously, these two flour characteristics have documented effects on specific volume of wheat-based products, and thus reasoning for this impact can also be applied to cell volume and diameter.

As corroborated by results for specific volume and crumb firmness, the small cell diameter and volume noted for breads produced from commercial flours are indications of the extreme density of the products. To further this hypothesis, Figure 4 illustrates the poor crumb structure of breads produced from commercial flours AF and TV. Gaps between the crust and crumb were nearly ubiquitous in bread samples produced from these flours. These flours clearly had a weak crumb structure that influenced oven collapse.

	Cell Diameter (mm)			Cell Volume (mm^3)		
	Pin Milling Treatment			**Pin Milling Treatment**		
	No	**Lo**	**Hi**	**No**	**Lo**	**Hi**
60%	2.23 ± 0.12^{Ab}	2.30 ± 0.14^{Ab}	2.35 ± 0.22^{Aab*}	8.00 ± 0.53^{Ab}	8.29 ± 0.67^{Abc}	8.61 ± 1.01^{Abc}
80%	2.48 ± 0.14^{Aa}	2.43 ± 0.17^{Ab}	2.44 ± 0.18^{Aab}	9.53 ± 0.65^{Aa}	9.29 ± 0.90^{Ab}	9.31 ± 0.90^{Ab}
100%	2.49 ± 0.15^{Aa}	2.68 ± 0.18^{Aa}	2.56 ± 0.21^{Aa}	10.28 ± 0.78^{Aa}	11.30 ± 1.07^{Aa}	10.63 ± 1.10^{Aa}
TV	2.41 ± 0.27^{Aa}	2.33 ± 0.10^{Ab}	2.33 ± 0.24^{Ab}	9.36 ± 1.38^{Aa}	8.93 ± 0.37^{Ab}	9.16 ± 1.27^{Ab}
AF	2.10 ± 0.15^{Ab}	2.07 ± 0.11^{Ac}	2.13 ± 0.21^{Abc}	7.68 ± 0.77^{Ab}	7.67 ± 0.48^{Ac}	8.20 ± 1.16^{Ac}

Table 10. Comparison of cell diameter and volume in bread produced from sorghum flours of varying extractions and pin-milling treatments

For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05).
For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05).
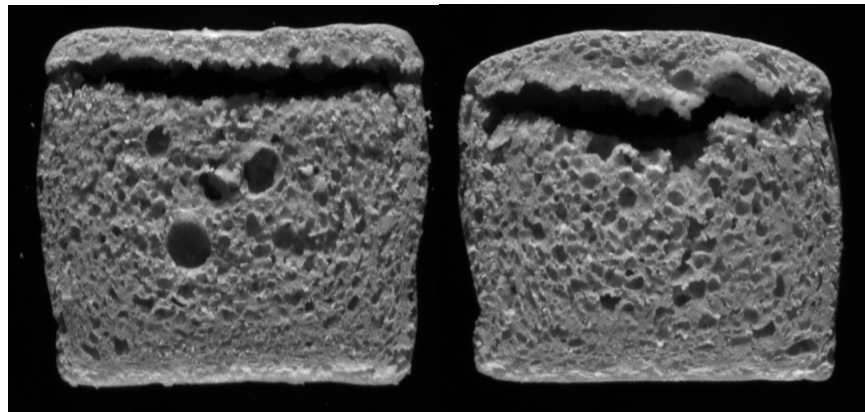*Indicates value is significant at p < 0.1.

Figure 4. C-Cell images. From left: Commercial
flour AF, No pin milling; Commercial Flour TV, no pin milling

Cells per Slice Area & Cell Wall Thickness

Results for measurements of cells per slice area and cell wall thickness in breads produced with all sorghum flours examined are shown in Table 11. Values for cells per slice area ranged from 40.03 cells/cm^2 (100% extraction, high-speed pin-milling) to 55.55 cells/cm^2 (AF, low-speed pin-milling). Values for cell wall thickness ranged from 0.489 mm (AF, low-speed pin-milling) to 0.557 mm (100% extraction, low-speed pin-milling). With the exception of breads produced with 100% extraction flour, pin-milling had no significant effect on either variable ($p<0.05$); bread produced from 100% extraction flour with high-speed pin-milling had a significantly lower number of cells per slice area and significantly thicker cells walls than low-speed or non pin-milled samples of the same extraction rate. Among all laboratory-milled samples, extraction level had no significant effect on number of cells per slice area or cell wall thickness. Within each pin-milling treatment, bread produced from both commercial flours exhibited the greatest number of cells per slice area and the thickest cell walls compared with breads produced from laboratory-milled flours. This, again, may be an indication of the density of these bread samples.

The ratio of cells per slice area is calculated by dividing the number of cells in each slice by the total slice area. The measurement attempts to provide standardization for variations in specific volume per loaf. However, this standardization effect has a tendency to diminish visible quality differences and should not be taken out of context. For example, the ratio of cells per slice area for bread produced from low-speed pin-milled 60% extraction flour is significantly lower than the ratio for AF with low-speed pin-milling, which numerically indicates a finer, more desirable crumb structure for the latter

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bread. However, as Figure 5 illustrates, bread produced from the 60% extraction flour has a distinct quality advantage and would be expected to be found more acceptable by consumers. As a second example, the ratios of cells per slice area for breads produced with 60% and 100% extraction flours with no pin-milling are not significantly different, indicating that the two breads do not differ in porosity. However, by examining Figure 6, it can again be seen that there are marked differences in crumb structure, the bread from 100% extraction bread being inferior. As such, it is this researcher's opinion that cells per slice area is not an accurate determinate of crumb quality for this particular study. It seems to be more appropriate for evaluating breads that are expected to have similar overall crumb characteristics, but slight differences in number of cells or slice area.

With that said, the measurement of cells per slice area may be able to provide some degree of insight into crumb structure. Variation in this ratio may be accompanied by a variation in cell wall thickness and cell diameter (and therefore, cell volume). In this study, the correlation coefficient between cells per slice area and cell wall thickness was -0.95 (Table 13); this could be interpreted to mean that breads with thicker cell walls were more likely to have a lesser amount of cells per standardized slice area, or vice versa. As such, cell diameter and cell volume may also be related to cells per slice area. In the present study, correlation coefficients between cells per slice area and cell diameter and cell volume were both -0.76. Additionally, cell diameter and cell volume correlated with cell wall thickness at coefficients of 0.78 and 0.84, respectively. Cell wall thickness has been shown to correlate with crumb grain character. Thin cell walls

predominate in fine-grained, fine-textured crumbs, and thicker cell walls are typically found in coarse-grained crumbs (Hayman and others 1998).

	Cells per Slice Area (cells/cm^2)			Cell Wall Thickness (mm)		
Table 11. Comparison of cells per slice area and cell wall thickness in bread produced from sorghum flours of varying extractions and pin-milling treatments						
	Cells per Slice Area (cells/cm^2)			Cell Wall Thickness (mm)		
	Pin Milling Treatment			Pin Milling Treatment		
	No	**Lo**	**Hi**	**No**	**Lo**	**Hi**
60%	44.99 ± 1.48^{Abc}	42.28 ± 2.30^{Abc}	43.26 ± 3.10^{Ab}	0.532 ± 0.015^{Aa}	0.545 ± 0.017^{Aa}	0.545 ± 0.018^{Aa}
80%	42.82 ± 1.91^{Ac}	42.53 ± 1.24^{Abc}	43.38 ± 3.10^{Ab}	0.555 ± 0.016^{Aa}	0.550 ± 0.014^{Aa}	0.547 ± 0.016^{Aa}
100%	45.54 ± 3.95^{Abc}	42.32 ± 5.48^{Abc}	40.03 ± 2.57^{Bbc}	0.541 ± 0.21^{ABa}	0.557 ± 0.024^{Aa}	0.562 ± 0.017^{Aa}
TV	48.85 ± 3.61^{Ab}	49.49 ± 1.33^{Ab}	46.53 ± 3.02^{Ab}	0.518 ± 0.21^{Ab}	0.517 ± 0.020^{Ab}	0.523 ± 0.020^{Ab}
AF	55.17 ± 3.01^{Aa}	55.55 ± 2.35^{Aa}	52.99 ± 4.88^{Aa}	0.492 ± 0.017^{Ac}	0.489 ± 0.014^{Ac}	0.498 ± 0.020^{Ac}

For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05).
For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05).

	Slice Area (mm^2)			Number of Cells (mm)		
Table 12. Comparison of slice area and number of cells in bread produced from sorghum flours of varying extractions and pin-milling treatments						
	Slice Area (mm^2)			Number of Cells (mm)		
	Pin Milling Treatment			Pin Milling Treatment		
	No	**Lo**	**Hi**	**No**	**Lo**	**Hi**
60%	3831 ± 189^{Aa}	4073 ± 181^{Aa}	3904 ± 152^{Aa}	1723 ± 83^{Aa}	1728 ± 81^{Aa}	1686 ± 73^{Aa}
80%	3528 ± 134^{Ab}	3703 ± 169^{Ab}	3504 ± 337^{Ab}	1511 ± 81^{Abc}	1574 ± 71^{Ab}	1514 ± 95^{Abc}
100%	3297 ± 182^{Ab}	3435 ± 319^{Abc}	3506 ± 142^{Ab}	1496 ± 59^{Abc}	1442 ± 96^{Ac}	1402 ± 83^{Ac}
TV	3247 ± 202^{Abc*}	3188 ± 245^{Ac}	3428 ± 203^{Ab}	1582 ± 86^{Ab}	1580 ± 153^{Ab}	1591 ± 67^{Aab}
AF	2969 ± 137^{Bc*}	3159 ± 149^{Abc}	3246 ± 275^{Ab}	1637 ± 78^{Bab}	1754 ± 85^{Aa}	1711 ± 94^{Aba}

For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05). For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05).
*Indicates value is significant at p < 0.1.

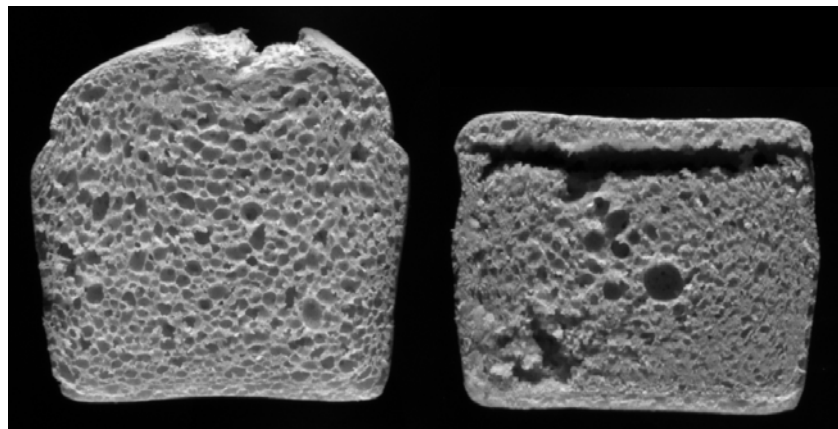| Table 13. Correlation coefficients between key crumb structure attributes.				
Variable	Cell Diameter	Cell Volume	Cells per Slice Area	Cell Wall Thickness
Cell Diameter	1.00	0.97	-0.76	0.84
Cell Volume	0.97	1.00	-0.76	0.78
Cells per Slice Area	-0.76	-0.76	1.00	-0.95
Cell Wall Thickness	0.84	0.78	-0.95	1.00

Figure 5. C-Cell images. From left: 60% Extraction, low-speed pin-milling; Commercial flour AF, low-speed pin-milling.
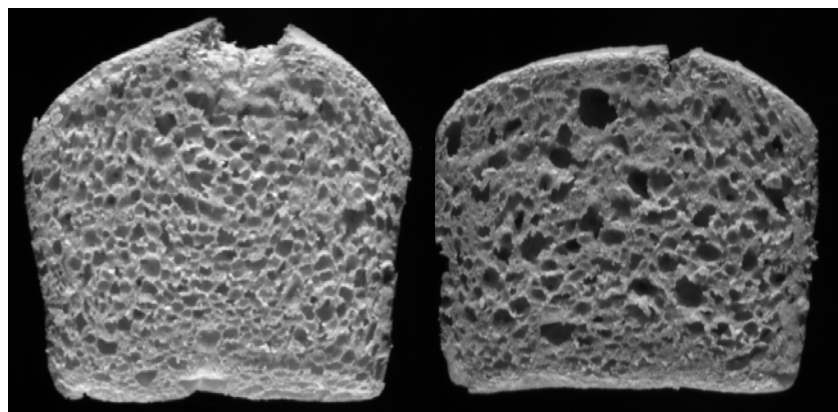

Figure 6. C-Cell images. From left: 60% Extraction, no pin-milling; 100% Extraction, no pin-milling.

Slice Brightness

Significant differences were found ($p<0.05$) for slice brightness of breads produced from all flour treatments studied (Table 14). Values ranged from 93.08 (100% extraction, high-speed pin-milling) to 127.97 (60% extraction, no pin-milling). For laboratory-milled flour samples, slice brightness significantly decreased with increased flour extraction ($p<0.05$). Higher extraction flours have a higher fiber content; a correlation coefficient of -0.94 ($p<0.0001$) was observed between slice brightness and fiber content. Higher extraction flours have a lower total starch content; a correlation coefficient of 0.82 ($p<0.0001$) was observed between slice brightness and total starch content. Per the results for L* and a* values for each flour treatment, it was discussed that fiber content impacted flour color. Sabanis and others (2009) hypothesized that crumb color is correlated to the color of the fiber. Additionally, Oh and others (1985) found that an increase in extraction rate caused a significant decline in brightness of noodles made with such flour.

Two bread samples showed significant differences in slice brightness within pin-milling treatment: breads produced with the high-speed pin-milled flours for the 100% extraction and AF showed significantly lower slice brightness values compared to the lower speed or non-pin-milled samples. Otherwise, no significant changes were observed for slice brightness due to pin-milling treatment. With that said, multiple linear regression did identify flour particle size as a significant predictor for slice brightness ($p<0.0001$).

As previously discussed, flour color is strongly correlated with crumb color (Pomeranz 1960). Together with texture, aroma, and flavor, color of baked products

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contributes to consumer preference (Sabanis 2009). Pyler (1988) noted that for white pan bread, the most desirable color is a soft, creamy white. However, in the previous discussion on flour color, the a* and b* values indicated that all sorghum flours studied had a grey appearance, rendering it unlikely that breads produced from these flours would appear as a soft, creamy white. Any opportunity to increase slice brightness in sorghum bread is recommended, and therefore, low extraction rate flours are recommended to obtain a more acceptable crumb color.

Table 14. Comparison of slice brightness values of crumb grain of bread produced from sorghum flours of varying extractions and pin-milling treatments.			
Slice Brightness			
Flour Extraction	**Pin-milling Treatment**		
	No	**Lo**	**Hi**
60%	127.97 ± 4.22^{Aa}	126.12 ± 2.97^{Aa}	127.42 ± 1.55^{Aa}
80%	115.44 ± 2.05^{Ab}	116.92 ± 0.61^{Ab}	114.78 ± 2.85^{Ab}
100%	96.46 ± 1.39^{Ad}	93.53 ± 2.08^{Bd}	93.08 ± 3.93^{Be}
TV	106.94 ± 3.27^{Ac}	107.04 ± 3.48^{Ac}	107.46 ± 3.73^{Ac}
AF	105.55 ± 2.24^{Ac}	104.24 ± 2.99^{Abc}	101.34 ± 3.03^{Bd}

For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05).
For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05).

TEXTURE PROFILE ANALYSIS

Crumb Firmness

Significant differences were found ($p < 0.05$) for firmness of bread slices (Table 15). Values ranged from 553.28 g (60% extraction, high-speed pin-milling) to 1096.26 g (AF, no pin-milling). Among laboratory-milled sorghum flours, there was an overall significant effect on bread texture from speed of pin-milling ($p < 0.05$) (Figure 8); high-speed pin-milled flours produced breads with the softest crumb texture within each extraction rate. There was a somewhat significant effect from extraction rate on bread texture; bread produced from 60% extraction flour had the softest crumb structure compared to 80% and 100% extraction flours, but the latter 2 samples were not considered significantly different from one another. Among all flour samples studied, bread produced from AF had the firmest bread texture, followed by TV which was significantly lower than AF, but significantly higher than either 80% or 100% extraction flour.

Firmness is a textural attribute associated with bread crumb and is defined as the resistance of the bread crumb to deformation (He and Hoseney 1990). Crumb firmness is a key attribute in baked goods, as it is strongly associated with consumers' perception of bread freshness (Ahlborn and others 2005). In white pan bread, most consumers prefer a soft, resilient, and short crumb (Pyler 1988). As breads produced from 60% extraction flour had the softest crumb, these flours are recommended for production of gluten-free sorghum bread. While it appears that a higher degree of pin milling does improve crumb texture, the effect of particle size and starch damage on other previously

discussed bread characteristics should be considered before deciding upon the ideal flour treatment.

Effect of Protein Content on Crumb Firmness

Previous researchers have determined that firming of wheat bread crumb is influenced by numerous variables, including protein and fiber content, moisture, baking temperature, and loaf volume (Ponte and others 1962; Maleki and others 1980; Moore and others 2004). Gluten-free bread has been shown to have much higher crumb firmness than other bread products. In a study by Ahlborn and others (2005), crumb firmness values for gluten-free rice bread were four times higher than for standard wheat or low-protein starch breads. In the present study, it appears that protein content had a somewhat significant effect on crumb firmness as it relates to extraction rate. Ramirez-Wong and others (2007) found that wheat flour tortillas produced with 100% extraction flour had the highest maximum stress among all flours studied, followed by 74% extraction flour. In a study on the incorporation of protein powders into gluten-free bread, Gallagher and others (2003) found that breads produced with more concentrated protein powders tended to have the firmest crumb compared to the control—produced with no additional protein. Additionally, Oh and others (1985) determined that internal firmness of cooked, hard wheat noodles was highly significant when correlated with protein content.

Effect of Fiber Content on Crumb Firmness

Increased fiber content is an outcome in flours with higher extraction rates. As such, the earlier reported observation from Ramirez-Wong and others (2007) showing an increase in firmness with an increase in extraction rate can partially be attributed to

fiber content as well. The present study showed that fiber content significantly contributed to firmness of bread ($p<0.0001$). Sabanis and others (2009) observed that fiber addition level significantly impacted crumb firmness of gluten-free bread at the $p<0.0001$ level. Gomez and others (2003) also reported an increase in crumb firmness upon the addition of wheat fiber into wheat bread. The researchers cited an explanation for increased firmness based upon the possible thickening of the cell wall due to fiber content. Another possible explanation for increased firmness in bread crumb due to fiber is due to increased gelatinization temperatures. The addition of pea hull, lentil, and chickpea fibers were found to cause an increase in the gelatinization of wheat breads (Dalgetty and Baik 2006; Santos and others 2008). Higher gelatinization temperatures have been shown to have association with a higher degree of starch crystallinity, which would increase bread firmness (Singh and others 2003).

Effect of Flour Particle Size on Crumb Firmness

Limited information exists on the effects of flour particle size and starch damage on the texture of bread, and especially gluten-free bread. However, in the present study, bread firmness significantly decreased for all extractions and flour types, so the effects of starch damage and/or flour particle size cannot be ignored. While statistical analysis showed that flour particle size did not significantly impact crumb texture, multiple linear regression revealed that starch damage was a significant predictor for crumb firmness ($p<0.0001$). However, again, changes in starch damage were caused by changes in particle size through pin-milling. Hatcher and others (2009) noted that both particle size and starch damage influenced white salted noodle quality. More specifically it was found that flours with fine particle size produced noodles with more acceptable textural

attributes than noodles produced from coarser flour. Finer particle size flours with higher degrees of starch damage may have experienced increased swelling, and therefore softening of the cooked noodles. Flour particle size was also noted to be a major contributing factor to tortilla texture (Wang and Flores 2000).

Effect of Loaf Volume on Crumb Firmness

Loaf volume also impacts crumb firmness. Sabanis and others (2009) noted a negative correlation between crumb firmness and loaf volume of -0.89 ($p < 0.05$). In the present study, the correlation between specific volume and firmness was -0.74. Similar results have also been reported (Gallagher and others 2003; He and Hoseney 1990). In each of these studies, including the present one, smaller loaves with lower specific volumes had denser and more tightly-packed crumb structures, resulting in higher values for crumb firmness. Indeed, crumb texture is affected by the cell structure; finer, thin-walled, uniformly sized cells produce breads with a softer and more elastic texture (Pyler 1988, Hayman and others 1998).

Table 15. Comparison of firmness of crumb of bread produced from sorghum flours of varying extractions and pin-milling treatments			
Firmness (g)			
Flour Extraction	Pin-milling Treatment		
	No	Lo	Hi
60%	771.01 ± 31.98^{Ad}	676.77 ± 33.51^{Bd}	553.28 ± 32.89^{Cd}
80%	$878.76 \pm 35.72^{Ac*}$	$761.05 \pm 36.11^{Bc*}$	711.72 ± 50.98^{Bc}
100%	936.37 ± 32.19^{Ac}	815.61 ± 53.77^{Bc}	748.32 ± 30.69^{Cc}
TV	943.05 ± 64.40^{Ab}	960.21 ± 57.82^{Ab}	846.41 ± 12.20^{Bb}
AF	1096.26 ± 52.06^{Aa}	1070.01 ± 62.89^{Aa}	1057.45 ± 60.13^{Aa}

For each <u>column</u>, mean values with the same <u>lowercase</u> superscript are not significantly different (p>0.05).
For each <u>row</u>, mean values with the same <u>uppercase</u> superscript are not significantly different (p>0.05).
*Indicates value is significant at p < 0.1.

CONCLUSION

Overall, this research demonstrates that sorghum flour composition and particle size affect the quality of gluten-free bread. To an extent, flours with lower amounts of fiber and a smaller particle size will produce breads with more acceptable characteristics, including volume, crumb structure, color, and texture. However, it is important to note that these flour characteristics do not exert their influences independently of one another. In fact, this research points to the importance in understanding the impact of starch damage on bread performance. This information may assist the milling industry in producing a more-value added sorghum flour, but will ultimately benefit consumers of gluten-free bread products.

RECOMMENDED FUTURE WORK

While foundations for studying gluten-free sorghum bread have been laid, questions still remain unanswered in the search for an increasingly acceptable product. One of the main outstanding issues is staling; sorghum breads stale more than twice as quickly as wheat bread (Hugo and others 1997). Investigating a delay in staling is essential in order for production of gluten-free sorghum breads to become commercialized as opposed to daily home baking. To this note, work is moving forward to investigate how enzymatic treatments of sorghum flour can improve baking quality, including a softer crumb structure and resistance to staling (Hugo and Taylor, unpublished data, as reported in Taylor and Dewar 2001).

Additionally, the results of this study suggest that sorghum flour with low fiber content may be favored for the production of gluten-free bread with acceptable volume. However, there are concerns about the sufficient incorporation of fiber into the gluten-free diet, as it is often filled with starch-based products lacking in complex carbohydrates and dietary fiber (Thompson and others 2005). Indeed, tracking of adults with celiac disease that follow a gluten-free diet has shown a lower daily intake of fiber than is recommended (Grehn and others 2001). As such, the incorporation of fiber into gluten-free bread would be invaluable to the celiac consumer. Much headway has been made in the baking industry with wheat bread formulations that include soluble fibers, such as fructooligosaccharides or resistant starches, and these technologies should be investigated for the development of gluten-free sorghum bread.

Finally, the inclusion of sensory testing into studies on the development of gluten-free products should be employed. There are challenges with this, as the typical bread

consumer has different quality expectations than a typical consumer of gluten-free products. Furthermore, recruitment of enough panelists to have a meaningful study remains a concern. However, having a better understanding about acceptance of current gluten-free bread will only continue to strengthen and improve future development of these products.

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